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     FILE 'MEDLINE, EMBASE, SCISEARCH, CAPLUS, USPATFULL' ENTERED AT 08:33:22
     ON 16 AUG 2000
L1         30825 S (UDP OR (URIDINE ADJ3 DIPHOSPHATE))
L2           648 S (ACETYLMURAMYLPENTAPEPTIDE OR (ACETYL MURAMYL PENTAPEPTIDE)
O
L3         35623 S (ACETYLGLUCOSAMINE OR (ACETYL GLUCOSAMINE) OR GLCNAC)
L4           120 S L1 AND L2 AND L3
L5         25751 S (PEPTIDOGLYCAN OR (GLYCAN))
L6           107 S L4 AND L5
L7            29 S L6 AND (BACTERI? (P) ENZYME?)
L8            31 S L6 AND ?ASSAY?
L9            17 S L7 AND ?ASSAY?
L10           13 S L6 AND TRANSGLYCOSYLASE?
L11            0 S TRANSLOCASE? AND TRANSFERASE? AND TRANSGLYCOSYLASE? AND
TRANS
L12            0 S L6 AND (LECTIN AND (BEAD? OR MICROBEAD? OR PARTICLE? OR MICR
L13            0 S L6 AND (LECTIN)
L14         1464 S L5 AND (LECTIN)
L15            0 S L14 AND L4
L16           64 DUP REM L6 (43 DUPLICATES REMOVED)
L17           25 DUP REM L7 (4 DUPLICATES REMOVED)
L18           21 DUP REM L8 (10 DUPLICATES REMOVED)
L19           17 DUP REM L9 (0 DUPLICATES REMOVED)
L20            5 DUP REM L10 (8 DUPLICATES REMOVED)
```

L19 ANSWER 1 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:144749 CAPLUS
DOCUMENT NUMBER: 132:191403
TITLE: Analogs of **UDP-MurNAc** peptides, **assays** and kits
INVENTOR(S): Axelrod, Helena R.; Branstrom, Arthur A.
PATENT ASSIGNEE(S): Incara Pharmaceuticals Corp., USA
SOURCE: PCT Int. Appl., 36 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000010587	A1	20000302	WO 1999-US18548	19990817
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9955636	A1	20000314	AU 1999-55636	19990817
PRIORITY APPLN. INFO.:			US 1998-97324	19980820
			WO 1999-US18548	19990817

REFERENCE COUNT: 7
REFERENCE(S): (1) Hoskins; US 5681694 A 1997 CAPLUS
(3) Ishiguro; J Bacteriol 1978, V135(3), P766 CAPLUS
(5) Tanaka; Biochim Biophys Acta 1977, V497(3), P633 CAPLUS
(6) Wickus; J Biol Chem 1972, V247(17), P5297 CAPLUS
(7) Zemell; J Biol Chem 1975, V250(8), P3185 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 2 OF 17 USPATFULL
ACCESSION NUMBER: 2000:61429 USPATFULL
TITLE: Auxiliary genes and proteins of methicillin resistant bacteria and antagonists thereof
INVENTOR(S): De Lencastre, Herminia, New York, NY, United States
Tomasz, Alexander, New York, NY, United States
PATENT ASSIGNEE(S): The Rockefeller University, New York, NY, United States
(U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 6063613	20000516	
	WO 9516039	19950615	
APPLICATION INFO.:	US 1995-403918	19950315	(8)
	WO 1994-US13952	19941206	
		19950315	PCT 371 date
		19950315	PCT 102(e) date

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1993-163053, filed on 6 Dec 1993, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Railey, II, Johnny F.

LEGAL REPRESENTATIVE: Klauber & Jackson
NUMBER OF CLAIMS: 3
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 17 Drawing Figure(s); 12 Drawing Page(s)
LINE COUNT: 2238
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L19 ANSWER 3 OF 17 USPATFULL
ACCESSION NUMBER: 2000:31197 USPATFULL
TITLE: Methods of screening for compounds active on Staphylococcus aureus target genes
INVENTOR(S): Benton, Bret, Burlingame, CA, United States
Lee, Ving J., Los Altos, CA, United States
Malouin, Francois, Los Gatos, CA, United States
Martin, Patrick K., Sunnyvale, CA, United States
Schmid, Molly B., Menlo Park, CA, United States
Sun, Dongxu, Cupertino, CA, United States
PATENT ASSIGNEE(S): Microcide Pharmaceuticals, Inc., Mountain View, CA, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6037123	20000314
APPLICATION INFO.:	US 1996-714918	19960913 (8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-3798	19950915 (60)
	US 1995-9102	19951222 (60)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Marschel, Ardin H.
LEGAL REPRESENTATIVE: Lyon & Lyon LLP
NUMBER OF CLAIMS: 60
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 91 Drawing Figure(s); 30 Drawing Page(s)
LINE COUNT: 16918
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L19 ANSWER 4 OF 17 USPATFULL
ACCESSION NUMBER: 2000:4670 USPATFULL
TITLE: Auxiliary genes and proteins of methicillin resistant bacteria and antagonists thereof
INVENTOR(S): Tomasz, Alexander, New York, NY, United States
De Lencastre, Herminia, New York, NY, United States
PATENT ASSIGNEE(S): The Rockefeller University, New York, NY, United States
(U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 6013507	20000111
APPLICATION INFO.:	US 1996-678614	19960710 (8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1995-1045	19950710 (60)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Elliott, George C.
ASSISTANT EXAMINER: Schwartzman, Robert
LEGAL REPRESENTATIVE: Klauber & Jackson
NUMBER OF CLAIMS: 7
EXEMPLARY CLAIM: 7
NUMBER OF DRAWINGS: 16 Drawing Figure(s); 20 Drawing Page(s)
LINE COUNT: 2405
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L19 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:281601 CAPLUS
DOCUMENT NUMBER: 133:101178
TITLE: **Assay** for Identification of Inhibitors for Bacterial MraY Translocase or MurG Transferase
AUTHOR(S): Branstrom, Arthur A.; Midha, Sunita; Longley, Clifford B.; Han, Kiho; Baizman, Eugene R.; Axelrod, Helena R.
CORPORATE SOURCE: Department of Biological Research, IRL, Inc., Cranbury, NJ, 08512, USA
SOURCE: Anal. Biochem. (2000), 280(2), 315-319
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 14
REFERENCE(S): (2) Brandish, P; Antimicrob Agents Chemother 1996, V40, P1640 CAPLUS
(5) Ikeda, M; J Bacteriol 1991, V173, P1021 CAPLUS
(7) Men, H; J Am Chem Soc 1998, V120, P2484 CAPLUS
(8) Mengin-Lecreulx, D; J Bacteriol 1991, V173, P4625 CAPLUS
(9) Mirelman, D; Biochem Biophys Res Commun 1972, V46, P1909 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L19 ANSWER 6 OF 17 USPATFULL
ACCESSION NUMBER: 1999:106323 USPATFULL
TITLE: Biosynthetic gene muri from Streptococcus pneumoniae
INVENTOR(S): Hoskins, Jo Ann, Indianapolis, IN, United States
Norris, Franklin Harpold, Indianapolis, IN, United States
Rockey, Pamela Kay, Indianapolis, IN, United States
Rosteck, Jr., Paul Robert, Indianapolis, IN, United States
Skatrud, Paul Luther, Indianapolis, IN, United States
Treadway, Patti Jean, Greenwood, IN, United States
Bellido, Michele Louise Young, Indianapolis, IN, United States
Wu, Chyun-Yeh Earnest, Indianapolis, IN, United States
PATENT ASSIGNEE(S): Eli Lilly and Company, Indianaplis, IN, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5948645	19990907	
APPLICATION INFO.:	US-1996-759907	19961204	(8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Caputa, Anthony C.
ASSISTANT EXAMINER: Navarro, Mark
LEGAL REPRESENTATIVE: Webster, Thomas D.
NUMBER OF CLAIMS: 10
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 751
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L19 ANSWER 7 OF 17 USPATFULL
ACCESSION NUMBER: 1999:43369 USPATFULL
TITLE: Metabolic pathway **assay**
INVENTOR(S): Chabin, Renee M., Neptune, NJ, United States
Kuo, David W., Princeton, NJ, United States
O'Connell, John F., Cranbury, NJ, United States
Pompliano, David L., Lawrenceville, NJ, United States

Wong, Kenny K., Edison, NJ, United States
PATENT ASSIGNEE(S): Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5891621	19990406
APPLICATION INFO.:	US 1997-936646	19970924 (8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-27331	19960930 (60)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Leary, Louise N.
LEGAL REPRESENTATIVE: Fitch, Catherine D.; Winokur, Melvin
NUMBER OF CLAIMS: 37
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 2 Drawing Figure(s); 2 Drawing Page(s)
LINE COUNT: 1247
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L19 ANSWER 8 OF 17 USPATFULL
ACCESSION NUMBER: 1998:138713 USPATFULL
TITLE: Biosynthetic gene murD from streptococcus pneumoniae
INVENTOR(S): Hoskins, Jo Ann, Indianapolis, IN, United States
Peery, Robert Brown, Brownsburg, IN, United States
Skatrud, Paul Luther, Indianapolis, IN, United States
Wu, Chyun-Yeh Earnest, Indianapolis, IN, United States
PATENT ASSIGNEE(S): Eli Lilly and Company, Indianapolis, IN, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5834270	19981110
APPLICATION INFO.:	US 1997-843309	19970414 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1996-665435, filed on 18 Jun 1996, now patented, Pat. No. US 5681694
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Wax, Robert A.
ASSISTANT EXAMINER: Stole, Einar
LEGAL REPRESENTATIVE: Webster, Thomas D.; Boone, David E.
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 764
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L19 ANSWER 9 OF 17 USPATFULL
ACCESSION NUMBER: 1998:124657 USPATFULL
TITLE: Biosynthetic gene murg from streptococcus pneumoniae
INVENTOR(S): Hoskins, Jo Ann, Indianapolis, IN, United States
Skatrud, Paul Luther, Indianapolis, IN, United States
Peery, Robert Brown, Brownsburg, IN, United States
PATENT ASSIGNEE(S): Eli Lilly and Company, Indianapolis, IN, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5821335	19981013
APPLICATION INFO.:	US 1996-751474	19961119 (8)

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Hutzell, Paula K.
ASSISTANT EXAMINER: Masood, Khalid
LEGAL REPRESENTATIVE: Webster, Thomas D.; Boone, David E.
NUMBER OF CLAIMS: 3
EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 764
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L19 ANSWER 10 OF 17 USPATFULL

ACCESSION NUMBER: 1998:124427 USPATFULL
TITLE: **Peptidoglycan** biosynthetic gene murE from Streptococcus pneumoniae
INVENTOR(S): Peery, Robert Brown, Brownsburg, IN, United States
Skatrud, Paul Luther, Indianapolis, IN, United States
PATENT ASSIGNEE(S): Eli Lilly and Company, Indianapolis, IN, United States (U.S. corporation)

	NUMBER	DATE
PATENT INFORMATION:	US 5821096	19981013
APPLICATION INFO.:	US 1997-818857	19970317 (8)

RELATED APPLN. INFO.: Division of Ser. No. US 1996-655114, filed on 29 May 1996, now patented, Pat. No. US 5712108, issued on 27 Jan 1998
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Wax, Robert A.
ASSISTANT EXAMINER: Stole, Einar
LEGAL REPRESENTATIVE: Webster, Thomas D.; Boone, David E.
NUMBER OF CLAIMS: 9
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 1 Drawing Figure(s); 1 Drawing Page(s)
LINE COUNT: 739
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L19 ANSWER 2 OF 17 USPATFULL

AB The present invention is directed to the identification of mutant strains of methicillin resistant bacteria, in particular methicillin resistant Staphylococcus aureus, to identify the characteristics of such bacteria and develop drugs that can reverse, inhibit or reduce bacterial resistance to beta lactam antibiotics, e.g., methicillin. The invention particularly relates to identification of a novel mutant strain of methicillin resistant S. aureus that manifests a unique phenotype. The mutant strain lacks unsubstituted pentapeptide and incorporates alanylglutamate- and alanylisoglutamine-containing muropeptides, and accumulates large amounts of the **UDP**-linked muramyul dipeptide in the cytoplasmic wall precursor pool of the mutant. Based on the phenotypic consequences of the mutation, inhibitors of the lysine addition step in bacterial cell wall biosyntheis are identified as having therapeutic potential for reducing bacterial resistance to beta lactam antibiotics, notably methicillin. Accordingly, the invention provides for methods of treatment and corresponding pharmaceutical compositions for treating bacterial, particularly staphylococcal, infections.

AB . . . unique phenotype. The mutant strain lacks unsubstituted pentapeptide and incorporates alanylglutamate- and alanylisoglutamine-containing muropeptides, and accumulates large amounts of the **UDP**-linked muramyul dipeptide in the cytoplasmic wall precursor pool of the mutant. Based on the phenotypic consequences of the mutation, inhibitors. . .

SUMM . . . a foreign source. The mecA gene encodes for a penicillin binding protein (PBP) called PBP2A (Murakami and Tomasz, 1989, J. **Bacteriol**. 171:874-79), which has very low affinity for the entire family of beta lactam antibiotics. In the current view, PBP2A is a kind of "surrogate" cell wall synthesizing **enzyme** that can take over the vital task of cell wall synthesis in staphylococci when the normal complement of PBPs (the. . . was a dramatic drop in resistance level from the minimum inhibitory concentration (MIC) value of 1600 .mu.g/ml in the parental **bacterium** to the low value of about 4 .mu.g/ml in the transposon mutant (Matthews and Tomasz, 1990, Antimicrobial Agents and Chemotherapy. . .

SUMM This observation is consistent with the foregoing theory. The mutant **bacteria** with their interrupted mecA gene could no longer synthesize PBP2A; thus the surrogate **enzyme** needed for the survival in the antibiotic-rich environment was no longer available to catalyze wall synthesis. Consequently, the methicillin susceptibility. . . the Tn551 mutant dropped to a level approaching the susceptibility of staphylococci without the mecA gene. Methicillin MIC for such **bacteria** is usually in the vicinity of 1-2 .mu.g/ml.

SUMM . . . functions. It was shown by a newly developed high resolution chromatography technique that many of the auxiliary mutants produced abnormal **peptidoglycan** in their cell walls. Studies combining High Performance Liquid Chromatography (HPLC) and mass spectrometry allowed the identification of the chemical. . . Jonge et al., 1992, J. Biol. Chem 267:11255-9; and De Jonge et al., 1993, J. Bacteriol. 175:2779-82). The cell wall **peptidoglycan** of auxiliary mutants was composed of muropeptides (cell wall building blocks) either with incomplete cross-linking peptides or containing a free. . .

SUMM . . . pentapeptide in the bacterial cell wall, and incorporation of alanylglutamate- and alanylisoglutamine-containing muropeptides, and

accumulation of large amounts of the **UDP**-linked muramyul dipeptide in the cytoplasmic wall precursor pool of the mutant bacteria.

SUMM . . . unsubstituted pentapeptide in the bacterial cell wall, incorporation of alanylglutamate- and alanylisoglutamine-containing muropeptides, and accumulation of large amounts of the **UDP** -linked muramyul dipeptide in the cytoplasmic wall precursor pool of the mutant bacteria.

SUMM In a specific embodiment, the invention contemplates reducing beta lactam antibiotic resistance in **bacteria** by administration of a competitive inhibitor antagonist of an **enzyme** or **enzymes** involved with addition of lysine to the dipeptide alanylisoglutamine and alanylglutamate, such as analogs of isoglutamine, analogs of glutamic acid, analogs of **UDP**-N-acetylmuramylalanylglutamate, and analogs of lysine.

DRWD FIG. 2. Suggested pathway for the addition of crosslinking peptides to the pentapeptide precursors. Symbols: G--N-**acetylglucosamine**; M--N-acetylmuramic acid; Ala, iGlu, Lys--alanine, isoglutamine and lysis, respectively. The synthetic pathway is interrupted at various steps in the auxiliary. . .

DRWD FIGS. 8A-C. HPLC elution profiles of muropeptides isolated from the parental strain, mutant RUSA235, and its backcross. **Peptidoglycan** was isolated and hydrolyzed with muramidase, and the resulting muropeptides were separated by HPLC as described under "Experimental Procedures." A:. . .

DRWD FIGS. 10A-B. Separation of cytoplasmic **peptidoglycan** precursors isolated from the parental strain and mutant RUSA235 by HPLC. Cytoplasmic precursors were isolated and separated by HPLC as. . .

DETD . . . active alleles in the parent bacterium, e.g., COL, chromosome; (C) the active alleles can be cloned into a shuttle-vector and **assayed** for the ability to complement, i.e., correct, the phenotype of the appropriate transposon mutant; and (D) the cloned gene or. . .

DETD (A) Cloning the insertionally inactivated (Tn551) form of auxiliary genes. 1. Digest the chromosomal DNA with different restriction **enzymes**, preferably selecting **enzymes** that cut once (or twice) inside Tn551, but that can be used for cloning in the plasmid to be used. . . (plasmid pRT1, see Matthews and Tomasz, 1990, Antimicrob. Agents Chemother. 34:1777-79) to find positive fragments--there will be two if an **enzyme** that cuts Tn551 once is used. 3. Elute the appropriate fragment or fragments identified with the probe from the gel.. . . E. coli vector (e.g., pUC19) and transform using an appropriate strain of E. coli as the recipient. 5. Select transformed **bacteria** in plates containing X-gal and IPTG; colonies containing recombinant plasmids will be white under these conditions. 6. Select the white. . .

DETD (C) Complementation **assay**. 1. The complementation **assay** involves the introduction of the recombinant plasmid putatively containing the inserted active allele of the auxiliary gene into the original. . .

DETD As described above, the presence of the gene may be detected by **assays** based on the physical, chemical, or immunological properties of its expressed product. For example DNA clones that produce a protein. . .

DETD . . . identified by the absence of the marker gene function. In the fourth approach, recombinant expression vectors can be identified by **assaying** for the activity of the gene product expressed by the recombinant. Such **assays** can be based, for example, on the physical or functional properties of the auxiliary gene product in suitable **assay** systems, e.g., cell wall synthesis.

DETD . . . (methicillin MIC=1600 mg/L), addition of 5-10 mg/L methicillin to the medium resulted in a striking change in the composition of **peptidoglycan** (de Jonge and Tomasz, 1993, Antimicrobial. Agents and Chemotherapy, 37:342-6). In drug free medium, this bacterium produces a cell wall. . . When grown in the methicillin containing medium, this complex wall structure is replaced by a simple one in which the **peptidoglycan** is made up of essentially two components; the pentaglycyl monomer and its dimer, with only a very small amount of trimers and traces of higher oligomers. Bacteria continue to produce this simple **peptidoglycan** throughout a vast range of antibiotic concentrations in the medium for 5 mg/L (<0.1% of the MIC) up to 750. . .

DETD Cell wall **peptidoglycan** can be prepared from parental strains and from mutants. The muropeptide building blocks of the **peptidoglycan** (liberated by enzymatic digestion) can be separated by reverse phase high performance liquid chromatography (HPLC) (de Jonge et al., 1992,. . . in the peptidoglycans, which can be identified by differences in HPLC elution profiles of muropeptides isolated from enzymatic cell wall **peptidoglycan** hydrolysates of a parental strain and of mutants (See e.g., de Jonge et al., J. Bacteriol. 173:1105-10; de Jonge et. . .

DETD In a specific embodiment, infra, mutation to a staphylococcal auxiliary gene results in a partial block in the cytoplasmic **peptidoglycan** precursor synthesis of the pentapeptide at the addition of the third (lysine) residue. This block is reflected in the appearance. . .

DETD . . . the lysine residue, and this monomer is also the major building block of dimers, trimers and higher oligomers of the **peptidoglycan**.

DETD . . . be selected from the group consisting of but not limited to analogs of isoglutamine, analogs of glutamic acid, analogs of **UDP**-N-acetylmuramylalanylglutamate, analogs of **UDP** -N-acetylmuramylalanylisoglutamine, and analogs of lysine. Such analogs are characterized by having the same topological structure, and therefore the same recognition features,. . .

DETD . . . with the structure of staphylococcal cell wall since previous studies have shown that the femA and B mutants had abnormal **peptidoglycan** crossbridge structures (5,6,7) and a femC mutant was shown to be blocked in the amidation of the alpha-carboxyl group of. . .

DETD . . . of antibiotic is consistent with this suggestion. The auxiliary mutants may indeed represent "methicillin-conditional" mutants in essential genes of staphylococcal **peptidoglycan** metabolism.

DETD Reduced Methicillin Resistance in a New Staphylococcus aureus Transposon Mutant that Incorporates Muramyldipeptides into the Cell Wall **Peptidoglycan**

DETD . . . value of the parent (1600 .mu.g/ml) to 25-50 .mu.g/ml in the mutant, caused heterogeneous expression of resistance, and abnormality of **peptidoglycan** composition: the unsubstituted pentapeptide was absent and alanyl-glutamate and alanyl-isoglutamate- containing muropeptides were incorporated in the cell wall. There was an accumulation of large amounts of the **UDP**-linked muramyl-dipeptide in the cytoplasmic wall precursor pool of the mutant. Both reduced (heterogeneous) antibiotic resistance and all the biochemical abnormalities. . .

DETD Preparation of the **UDP**-linked precursor and analysis with HPLC. Cytoplasmic pools of **UDP**-linked **peptidoglycan** precursor were extracted by a modification of a previously described method (Handwerger etal., 1994, J. Bacteriol 176:260-264). Cells were grown. . . separated by gel filtration on a Sephadex G-25 column

(Pharmacia, Alameda, Calif.) eluted with water. Hexosamine-containing fractions identified by the **assay** of Ghuysen et al. (1966, Methods Enzymol. 8:684-699) were combined and lyophilized. Separation of the muropeptides by HPLC was performed. . . .mu.g/ml) were used as reference for determination of retention times of the tripeptide and pentapeptide precursor compounds. The major uridine-diphosphate (**UDP**) containing peak from the cytoplasmic precursor extract of RUSA235 was collected, desalted and analyzed for amino acid composition.

UDP-N-acetylglucosa- mine from Sigma (St. Louis, Mo.) was used as a standard. Boiling samples for 3 min in 0.1 M HCl before loading onto the column was used to identify **UDP**-containing peaks.

DETD Muropeptide composition of the **peptidoglycan** of RUSA235 as determined by HPLC and chemical analysis. The HPLC elution profiles for the parental strain COL, RUSA235 and. . .

DETD TABLE 6

Amino acid analysis and molecular masses of the anomalous muropeptides isolated from the muramidase digest of the **peptidoglycan** of mutant RUSA23S

HPLC					Mass
Muro- peptide.sup.a	retention time min	Amino acid analysis Glx	Ala	Lys Gly	spectrometry (M + H).sup.+c

a. . .

DETD **UDP**-lined cell wall precursor pool of parental strain COL and mutant RUSA235. FIG. 10 shows the HPLC elution profiles of **UDP** -linked precursors extracted from the parental and mutant staphylococci.

Table 7 shows that quantitative differences between the composition of parental and. . . pool. Peak IV, a component absent from the precursor pool of parental cells, has accounted for over 60% of the **UDP**-linked muropeptides in the mutant extract. This material was isolated and identified as **UDP**-N-acetyl-muramyl-alanyl-glutamate on the basis of chemical analysis (UV spectra, Elson-Morgan reaction and quantitative amino acid analysis).

DETD TABLE 7

UDP-linked **peptidoglycan** of mutant RUSA235 and its parental strain (COL)

The relative amounts of the compounds (peaks I-VI) are expressed as percentages (calculated from the UV absorbance of peaks in HPLC elution profiles). Data represent the means of three experiments.

UDP-GlcNAc, uidine diphospho-N-**acetylglucosamine** ;

UDP-MurNAc, uridine diphospho-N-acetylmuramic acid, Ala, L-alanine;

Glu, D-glutamate; Lys, L-lysine; Penta, L-alanine:D-glutamate:L-lysine:D-alanine;D-alanine.

I	II	III	IV	V	VI	Total
	UDP-GlcNAc	**UDP-MurNAc**	**UDP**			
	-Mur-Ala	**UDP**-Mur-Ala-Glu	**UDP**-Mur-Ala-Glu-Lys			
						UDP
	-Mur-Penta					
						hexosamines
Strain	%	%	%	%	%	% nmol
COL	20 .+-. 2					
	28 .+-. 3					
		41 .+-. 3				
			0	0. . .		

DETD . . . of a library of Tn551 mutants of MRSA for reduced methicillin

resistance has already identified several transposon mutants with altered **peptidoglycan** composition, either in the stem peptides or in the crossbridges. Previously described mutants located outside the mecA gene had transposon. . .

DETD The **peptidoglycan** of RUSA235 is composed of the same muropeptide species as the parental strain in the same proportions and same degree. . . is alanine and the amino acid at the second position is either isoglutamine or glutamic acid. Analysis of the cytoplasmic **peptidoglycan** precursor pool of RUSA235 revealed an accumulation of the **UDP**-linked muramyl-dipeptide containing equimolar amounts of alanine and glutamic acid and reduced level of the **UDP**-linked muramyl-pentapeptide. These data indicate that the RUSA235 mutation is in a gene (femF) responsible for the biosynthetic step in which. . . route at this step may explain the observed deficit in muropeptide species which contain unsubstituted pentapeptide units in the mutant **peptidoglycan**.

DETD Some of the properties of RUSA235 are reminiscent of a S. aureus conditional mutant TOF-95 (Good and Tipper, 1972, J. **Bacteriol** 111:231-241) and RUS 1 (Chatterjee and Young, 1972, J. **Bacteriol** 111:220-230), both of which are defective in cell wall precursor synthesis which also showed accumulation of **UDP**-linked muramyl dipeptide cell wall precursors and a defective lysine adding **enzyme**. However, in contrast to TOF-95, RUSA235 was capable of growth at elevated temperature (43.degree. C.) without osmotic supplementation of the. . .

DETD . . . lacking the diaminoacid component (and thus unable to participate in crosslinking) may incorporate into some structurally critical positions in the **peptidoglycan** and this may, indirectly, jeopardize the integrity of wall structure during perturbation of wall synthesis by antibiotics.

DETD 5. De Jonge, B. L. M., Y. -S. Chang, D. Gage, and A. Tomasz. 1992. **Peptidoglycan** composition of a highly methicillin-resistant Staphylococcus aureus strain: the role of penicillin binding protein 2A. J. Biol. Chem. 267:11248-11254.

DETD 6. De Jonge, B. L. M., Y. -S. Chang, D. Gage, and A. Tomasz. 1992. **Peptidoglycan** composition in heterogeneous Tn551 mutants of a methicillin resistant Staphylococcus aureus strain. J. Biol. Chem. 267:11255-11259.

DETD 13. Henze, U., T. Sidow, J. Wecke, H. Labischinski, and B. Berger-Bachi. 1993. Influence of femB on methicillin resistance and **peptidoglycan** metabolism in Staphylococcus aureus. J. Bacteriol. 175:1612-1620.

DETD 17. Ornelas-Soares, A., H. de Lencastre, B. de Jonge, D. Gage, Y. -S. Chang, and A. Tomasz. 1993. The **peptidoglycan** composition of a Staphylococcus aureus mutant selected for reduced methicillin resistance. J. Biol. Chem. 268:26268-26272.

DETD These data indicate that interference with the **peptidoglycan** biosynthesis at the cytoplasmic level affects methicillin resistance in S. aureus.

CLM What is claimed is:

. . . having the structure alanylglutamate and alanylisoglutamine and lacking lysine, lack of unsubstituted pentapeptide in the bacterial cell wall, and accumulating **UDP**-N-acetylmuramylalanylglutamate in the precursor pool.

L19 ANSWER 3 OF 17 USPATFULL

AB This disclosure describes isolated or purified deoxyribonucleotide (DNA) sequences, useful for the development of antibacterial agents, which contain the coding sequences of bacterial pathogenesis genes or

essential genes, which are expressed in vivo. It further describes isolated or purified DNA sequences which are portions of such bacterial genes, which are useful as probes to identify the presence of the corresponding gene or the presence of a bacteria containing that gene. Also described are hypersensitive mutant cells containing a mutant gene corresponding to any of the identified sequences and methods of screening for antibacterial agents using such hypersensitive cells. In addition it describes methods of treating bacterial infections by administering an antibacterial agent active against one of the identified targets, as well as pharmaceutical compositions effective in such treatments.

SUMM . . . the context of compounds, agents, or compositions having antibacterial activity indicates that the compound exerts an effect on a particular **bacterial** target or targets which is deleterious to the in vitro and/or in vivo growth of a **bacterium** having that target or targets. In particular, a compound active against a **bacterial** gene exerts an action on a target which affects an expression product of that gene. This does not necessarily mean. . . of the gene has a major biological role. Consequently, such a compound can be said to be active against the **bacterial** gene, against the **bacterial** gene product, or against the related component either upstream or downstream of that gene or expression product. While the term. . . also implies some degree of specificity of target. Therefore, for example, a general protease is not "active against" a particular **bacterial** gene which produces a polypeptide product. In contrast, a compound which inhibits a particular **enzyme** is active against that **enzyme** and against the **bacterial** gene which codes for that **enzyme**.

SUMM . . . binding with approximately 85% sequence identity). The equivalent function of the product is then verified using appropriate biological and/or biochemical **assays**.

SUMM . . . of the expression product. Such methods can include, for example, antibody binding methods, enzymatic activity determinations, and substrate analog binding **assays**.

SUMM It is quite common in identifying antibacterial agents, to **assay** for binding of a compound to a particular polypeptide where binding is an indication of a compound which is active. . .

DETD . . . both the nucleic acid and protein levels reveal identity to the S. aureus femA gene, encoding a protein involved in **peptidoglycan** crosslinking (Genbank Accession No. M23918; published in Berger-Baechi, B., et al., Mol. Gen. Genet. 219, (1989) 263-269). The pMP33 clone. . .

DETD . . . both the nucleic acid and protein levels reveal identity to the Staph. aureus femB gene, encoding a protein involved in **peptidoglycan** crosslinking (Genbank Accession No. M23918; published in Berger-Baechi, B., et al., Mol. Gen. Genet. 219, (1989) 263-269). The pMP40. . .

DETD . . . proteins FemA and FemB, suggesting that clone pMP55 contains a new Fem-like protein. Since the Fem proteins are involved in **peptidoglycan** formation, this new Fem-like protein is likely to make an attractive candidate for screening antibacterial agents. Since clone pMP55 does. . .

DETD . . . both the nucleic acid and peptide levels reveal strong similarities at the peptide level to the murC gene product, encoding **UDP**-N-Acetyl muramoyl-L-alanine synthase (EC 6.3.2.8), from B. subtilis (Genbank Accession No. L31845).

DETD . . . at both the nucleic acid and peptide levels reveal strong similarity at the peptide level to the murG gene, encoding **UDP** -**GlcNAc**:undecaprenyl-pyrophosphoryl-pentapeptide transferase, from B. subtilis (Genbank Accession No. D10602; published in Miyao, A. et al. Gene 118 (1992) 147-148.) Cross complementation. . .

DETD . . . acid and (putative) polypeptide levels against currently available databases reveal strong peptide-level similarity to ylxC,

encoding a putative murB homolog (**UDP-N-**
acetylenolpyruvoylglucosamine reductase), in B. subtilis (Genbank Accession No. M31827). The predicted relative size and orientation of the ylxC gene is depicted. . .

DETD . . . (putative) polypeptide levels against currently available databases reveal significant similarities to the murD gene product from B. subtilis, which encodes **udp-MurNAc**
-dipeptide::D-Glu ligase (EC 6.3.2.9); similarities are also noted to the equivalent gene products from E. coli and H. influenzae. The predicted. . .

DETD . . . the function of the product of a specific host gene is known, homologous gene products can often be isolated (by **assaying** for the appropriate activity) and at least partially sequenced (e.g., N-terminal sequencing). The amino acid sequence so obtained can then.

.

DETD . . . models are available and may be used when appropriate for specific pathogens or specific genes. For example, target organ recovery
assays (Gordee et al., 1984, J. Antibiotics 37:1054-1065; Bannatyne et al., 1992, Infect. 20:168-170) may be useful for fungi and for. . .

DETD . . . virulence may be employed (Falkow et al., 1992, Ann. Rev. Cell Biol. 8:333-363). These include, but are not limited to, **assays** which measure bacterial attachment to, and invasion of, tissue culture cell monolayers. With specific regard to S. aureus, it is. . .

DETD . . . use of the properties of the crippled gyrase mutants in a screen provides a great advantage over biochemical-based screens which **assay** a single specific function of the target protein in vitro.

DETD . . . will not be useful in semi-permissive growth conditions. Such mutant alleles may have nearly wild type function at the screening **assay** temperature. The simplest method for validating the use of ts mutants is to select those which show a reduced growth. . . partially defective. More specific methods of characterizing the partial
defect of a mutant strain are available by biochemical or physiological **assays**.

DETD . . . FIG. 4. In this screen design, one plate serves to evaluate one
compound. Each well provides a separate whole-mutant cell **assay** (i.e., there are many targets per screening plate). The **assays** are genetic potentiation in nature, that is, ts-hypersensitive mutants reveal compounds that are growth inhibitors at concentrations that do not. . .

DETD The use of the 96-well multi-channel screen format allows up to 96 different **assays** to characterize a single compound. As shown in FIG. 5, this format provides an immediate characterization or profile
of a single compound. The more traditional format, using up to 96 different compounds per plate, and a single **assay** can also be readily accommodated by the genetic potentiation **assays**.

DETD . . . plate, cross comtamination between different strains and the testing of different mutants at different temperatures (or with other changes in **assay** conditions) are no longer problems. Moreover, this strategy retains the same compound arrangement in all compound plates, thus saving time,. . .

DETD . . . a particular compound will fail to grow. Thus, even compounds considered "generally toxic" should show some specificity of action, when **assayed** with the hypersensitive mutant strains.

DETD b. Compounds that affect few (or no) mutants. Since all compounds **assayed** in the preliminary screen inhibit the growth of the wild type strain to some degree (initial basis of pre-selection), such. .

.

DETD . . . at critical points in the metabolic web. Still other mutants may have specific alleles that are highly crippled at the **assay** temperature. For these mutants, the metabolic web consequences are large

because the specific allele has created a highly hypersensitive strain.

DETD . . . true specificity that was yet not revealed by any compound, or that these mutants have nearly full activity at the **assay** temperature. This analysis stresses the importance of strain validation as indicated above.

DETD Genetic potentiation **assays** provide a rapid method to implement a large number of screens for inhibitors of a large number of targets. This. . .

DETD . . . the gene clones, selection of resistant alleles provides early evidence for the specific target. Subsequent efforts to establish a biochemical **assay** for rapid, specific and sensitive tests of derivative compounds will be aided by the over-expression and purification of the target. . . comparing the mutant and wild type strain to confirm the novel target function, and aid in the establishment of biochemical **assays** for the targets.

DETD d. Compound Binding and Molecular Based **Assays** and Screens

DETD Alternatively, once the identity of a polypeptide is known, and an **assay** for the presence of the polypeptide is determined, the polypeptide can generally be isolated from natural sources, without the necessity for a recombinant coding sequence. Such **assays** include those based on antibody binding, enzymatic activity, and competitive binding of substrate analogs or other compounds. Consequently, this invention. . .

DETD For use of binding **assays** in screening for compounds active on a specific polypeptide, it is generally preferred that the binding be at a substrate. . .

DETD Binding **assays** can be provided in a variety of different formats. These can include, for example, formats which involve direct determination of. . . a change in a relevant activity, and formats which involve competitive binding. In addition, one or more components of the **assay** may be immobilized to a support, though in other **assays**, the **assays** are performed in solution. Further, often binding **assays** can be performed using only a portion of a polypeptide which includes the relevant binding site. Such fragments can be. . . the art can also be used. Thus, essential genes identified herein provide polypeptides which can be utilized in such binding **assays**. Those skilled in the art can readily determine the suitable polypeptides, appropriate binding conditions, and appropriate detection methods.

DETD . . . product of an essential gene can also allow use of a molecular based (i.e., biochemical) method for screening or for **assays** of the amount of the polypeptide or activity present in a sample. Once the biological activities of such a polypeptide are identified, one or more of those activities can form the basis of an **assay** for the presence of active molecules of that polypeptide. Such **assays** can be used in a variety of ways, for example, in screens to identify compounds which alter the level of activity of the polypeptide, in **assays** to evaluate the sensitivity of the polypeptide to a particular compound, and in **assays** to quantify the concentration of the polypeptide in a sample.

DETD . . . as the ratio LD.sub.50 /ED.sub.50. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture **assays** and animal studies can be used in formulating a range of dosage for use in human. The dosage of such. . .

DETD . . . any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture **assays**. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the. . .

L19 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2000 ACS

AB Bacterial **peptidoglycan** synthesis is a well-characterized system for targeting new antimicrobial drugs. Formation of the **peptidoglycan** precursors Lipid I and Lipid II is catalyzed by the

gene products of mraY and murG, which are involved in the first and second steps of the lipid cycle reactions, resp. Here we describe the development of an **assay** specific for identifying inhibitors of MraY or MurG, based on the detection of radiolabeled [14C]**GlcNAc** incorporated into Lipid II. **Assay** specificity is achieved with the biotin tagging of the Lipid I precursor **UDP-MurNAc** -pentapeptide. This allows for the sepn. and identification of lipid products produced by the enzymic activity of the MraY and MurG proteins, and thus identification of specific inhibitors. (c) 2000 Academic Press.

TI **Assay** for Identification of Inhibitors for Bacterial MraY Translocase or MurG Transferase

AB Bacterial **peptidoglycan** synthesis is a well-characterized system for targeting new antimicrobial drugs. Formation of the **peptidoglycan** precursors Lipid I and Lipid II is catalyzed by the gene products of mraY and murG, which are involved in the first and second steps of the lipid cycle reactions, resp. Here we describe the development of an **assay** specific for identifying inhibitors of MraY or MurG, based on the detection of radiolabeled [14C]**GlcNAc** incorporated into Lipid II. **Assay** specificity is achieved with the biotin tagging of the Lipid I precursor **UDP-MurNAc** -pentapeptide. This allows for the sepn. and identification of lipid products produced by the enzymic activity of the MraY and MurG. . .

ST translocase MraY transferase MurG inhibitor **assay**

IT Transport proteins
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(MraY; **assay** for identification of inhibitors of bacterial MraY translocase or MurG transferase)

IT Glycopeptides
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(muramic acid-contg., **UDP**-Mur-NAc-pentapeptide, biotinylated; **assay** is based on transfer of N-acetyl-D-glucosamine from uridine-diphosphate)

IT Peptidoglycans
RL: MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative)
(precursor, Lipid II; **assay** is based on transfer of N-acetyl-D-glucosamine from uridine-diphosphate)

IT 60976-26-3, Gene MurG **enzyme**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(MurG; **assay** for identification of inhibitors of **bacterial** MraY translocase or MurG transferase)

IT 528-04-1
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**assay** for identification of inhibitors of bacterial MraY translocase or MurG transferase)

IT 7512-17-6, N-**Acetyl-glucosamine**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**assay** is based on transfer of N-acetyl-D-glucosamine from uridine-diphosphate)

L19 ANSWER 7 OF 17 USPATFULL

AB An in vitro screening **assay** which identifies enzyme inhibitors and allows for the simultaneous **assay** of many enzymes. Enzyme, substrate, co-factor, etc. concentrations are optimized so that inhibitors of any one of the enzymes in the pathway are equally likely to be detected. Necessarily, the flux of substrate through each enzyme should be nearly the same during the **assay**, i.e., each of the enzyme catalyzed steps must be equally rate-limiting. Preferably, optimal **assay** conditions are predicted by computer modeling. Further, the pathway conditions are optimized through variation of enzyme, starting substrate, co-substrate and co-factor concentrations.

A

positive response is initially detected as a change in the amount of the product generated at the end of the enzyme cascade as compared to a standard. A sample producing a positive result can be deconvoluted.

TI Metabolic pathway **assay**

AB An in vitro screening **assay** which identifies enzyme inhibitors and allows for the simultaneous **assay** of many enzymes. Enzyme, substrate, co-factor, etc. concentrations are optimized so that inhibitors of any one of the enzymes in. . . equally likely to be detected. Necessarily, the flux of substrate through each enzyme should be nearly the same during the **assay**, i.e., each of the enzyme catalyzed steps must be equally rate-limiting. Preferably, optimal **assay** conditions are predicted by computer modeling. Further, the pathway conditions are optimized through variation of enzyme, starting substrate, co-substrate and. . .

SUMM This invention relates to an in vitro screening **assay** which identifies enzyme inhibitors. This invention allows for the simultaneous **assay** of many enzymes. The goal is to optimize the concentrations of enzymes and substrates so that inhibitors of any one. . . In order for this to occur, the flux of substrate through each enzyme should be nearly the same during the **assay**, i.e., each of the enzyme catalyzed steps must be equally rate-limiting. Consequently, optimal **assay** conditions can be predicted, preferably by mathematical modeling. Further, the pathway conditions are optimized through variation of enzyme, starting substrate,. . . enzyme cascade as compared to a standard. A sample producing a positive result can be deconvoluted. Additionally, the metabolic pathway **assay** of the present invention reduces the labor involved in enzyme **assay**: because it is pathway **assay**, only the initial substrate need be prepared.

SUMM One aspect of the present invention is an in vitro screening **assay** for a biologically active compound, which is comprised of an enzyme cascade comprising a first enzyme, a second enzyme and. . .

SUMM Additionally, further embodiments of the present invention include, but are not necessarily limited to, any enzyme cascade **assay** or method of using said **assay** to identify biologically active compounds, wherein the cascade is comprised of any sequential combination of the enzymes which comprise the. . .

SUMM . . . involving disease or pathways unique to pathogens may have useful bioactivity. Therefore, it is desirable to identify such inhibitors. Currently, **assays** of complete metabolic pathways in vitro are complicated by the kinetics of individual enzymes. Heretofore, component enzymes of a particular pathway were individually purified and **assayed** one by one. This individual **assay** approach also involved making the substrate for each enzyme separately. Further, attempts have been made to model behavior of metabolic. . . used thus far only to study metabolic processes, has drawbacks as well. Specifically, one metabolic step is rate-limiting; therefore, when **assaying** using such a pathway model, it is more likely to find an inhibitor for one particular enzyme.

SUMM In particular, the present invention can be applied to the murein biosynthetic pathway. Compounds that inhibit **enzymes** along this pathway are expected to be antibiotics. Each gene in that pathway (murABCDEFGI, mraY, ddlA, alr) is essential for **bacterial** viability. The pathway is uniquely **bacterial**: no known eukaryotic homologues of these genes exist. There are known antibiotics (fosfomycin, cycloserine) whose molecular target is within the pathway. Additionally, this pathway is highly conserved amongst pathogenic **bacteria**, and thus it is expected that an inhibitor of this pathway will be a broad spectrum antibiotic.

DETD The present invention, a metabolic pathway **assay**, relates to an in vitro screening **assay** which identifies biologically active compounds, namely enzyme inhibitors. The present invention further relates to methods of identifying biologically active compounds

using a pathway **assay**.

DETD Generally, the metabolic pathway **assay** of the present invention allows for the simultaneous **assay** of many enzymes. In the present invention, metabolic pathways are reconstructed from isolated, preferably purified, enzymes, substrate, co-substrates, co-factors, buffers,. . .

DETD One aspect of the present invention is an in vitro screening **assay** for a biologically active compound, which is comprised of an enzyme cascade comprising a first enzyme, a second enzyme and. . .

DETD A second embodiment of the invention is an in vitro screening **assay** additionally comprising one or more co-substrates for the first and second enzymes.

DETD A third embodiment of the invention is an in vitro screening **assay** for a biologically active compound which is comprised of an enzyme cascade comprising a first enzyme, a second enzyme, a. . .

DETD A fourth embodiment of the invention is an in vitro screening **assay** for a biologically active compound which is comprised of an enzyme cascade comprising a first enzyme, a second enzyme, a. . .

DETD A species of this subclass is one in which the substrate for the first enzyme is uridyl-5'-diphosphate-N-**acetyl-glucosamine**; the co-substrate for the first enzyme is phosphoenolpyruvate; the substrate for the second enzyme is uridyl-5'-diphosphate-N-acetyl-enolpyruvylglucosamine; the co-substrates for the. . .

DETD . . . of this subclass is a method comprised of an enzyme cascade in which the substrate for the first enzyme is uridyl-5'-diphosphate-N-**acetyl-glucosamine**; the co-substrate for the first enzyme is phosphoenolpyruvate; the substrate for the second enzyme is uridyl-5'-diphosphate-N-acetyl-enolpyruvylglucosamine; the co-substrate for the. . .

DETD Additionally, further embodiments of the present invention include, but are not necessarily limited to, any enzyme cascade **assay** or method of using said **assay** to identify biologically active compounds, wherein the cascade is comprised of any sequential combination of the enzymes which comprise the. . .

DETD . . . co-substrates and co-factors may be necessary for the above-mentioned embodiments. Such materials include, but are not necessarily limited to: uridyl-5'-diphosphate N-**acetyl-glucosamine** (UDPAG); phosphoenolpyruvate; uridyl-5'-diphosphate-N-acetyl-enolpyruvylglucosamine; nicotinamide adenine dinucleotide phosphate reduced form (NADPH); flavin adenine dinucleotide (FAD); uridyl-5'-diphosphate-N-acetyl-muramic acid (UDPMurNAc); L-alanine; ATP;

L-glutamic acid; UDPMurNAc-L-alanine; D-glutamic acid; UDPMurNAc-L-alanyl-.gamma.-D-glutamic acid; meso-diaminopimelic acid; UDPMurNAc-L-alanyl-.gamma.-D-glutamyl-meso-diaminopimelic acid; D-alanyl-D-alanine; UDPMurNAc-L-alanyl-.gamma.-D-glutamyl-meso-diaminopimelyl-D-alanyl-D-alanine; undecaprenyl diphosphate; and undecaprenyl-diphosphoryl-**MurNAc**-L-alanyl-.gamma.-D-glutamyl-meso-diaminopimelyl-D-alanyl-D-alanine.

DETD The **enzymes** which comprise a particular pathway may be obtained in a number of ways. First, **enzyme**-encoding gene sequences can be used to make **enzymes** for the **assay**. Each gene is cloned by PCR, or polymerase chain reaction. The genes are expressed using commercially available expression vectors or. . .

can

be, but is not necessarily, accomplished via glutathione-S-transferase (GST), maltose binding protein (MBP), or other similar fusions. The expressed **enzymes** are then purified. If the **enzymes** were expressed by protein fusion, the **enzymes** are purified by affinity chromatography specific to the fusion protein used. The **enzymes** may then be cleaved from the protein with a suitable protease. Both free **enzyme** and protein-fused **enzymes** can be used in the **assay** of the present invention. Second, native **enzymes** may be isolated from **bacterial** cells.

Alternatively, **enzymes** comprising the pathway to be **assayed** may be purchased if commercially available.

DETD . . . embodiment of the present invention, the known gene sequences for murC, murD, murE and murF are used to synthesize their **enzyme** products: **UDP**-N-acetylmuramoyl: L-alanine ligase; **UDP**-N-acetylmuramoyl- L-alanine: D-glutamate ligase; **UDP**-N-acetylmuramoyl-L-alanyl-D-glutamate: meso-2,6-diaminopimelate ligase; and **UDP**-N-acetylmuramoyl-L-alanyl-D-glutamyl-meso-2,6-diaminopimoyl-D-alanine-D-alanine synthase, respectively. The genes are cloned by PCR and expressed using a modified commercially available GST or MBP fusion. . . are referenced under Hakes, D. J. and Dixon, J. E. "New Vectors for High Level Expression of Recombinant Proteins in **Bacteria**" 202 Analytical Biochemistry 293-298 (1992). The protein expression is detailed in Reference Example 5. The expressed **enzymes** are purified by affinity chromatography specific to the fusion protein used in the expression or any other suitable purification method.. . . GST fusions bind to glutathione agarose columns eluted with glutathione. MBP fusions bind to amylose columns eluted with maltose. The **enzyme** is cleaved from the purified fusion protein by incubating with thrombin. The contaminating GST or MBP can be removed by passage of the thrombin cleavage reaction mixture through the glutathione or amylose column one more time. The free **enzyme** passes through without binding to the column, while the GST or MBP will specifically stick to the column. The purification of the **enzyme** products of murC, murD, murE and murF is detailed in Reference Examples 6-9. In one embodiment of the present invention, the free mur **enzymes** are used for pathway **assay**. In another embodiment of the present invention, GST or MBP fusions of the mur **enzymes** are used for pathway **assay**. In an embodiment of the invention, MBP fusions are utilized.

DETD The pathway **assay** also may contain a biological buffer, which maintains the requisite pH level for the specific enzyme-catalyzed reactions. Any buffer of. . .

DETD The **assay** also may contain a marker or tag that is useful for detection and deconvolution purposes. For instance, any suitable radioactive. . .

DETD The **assay** also may contain a number of co-substrates. By the term "co-substrate" is meant any agent that is metabolized and is. . .

DETD The **assay** also may contain a number of co-factors. By the term "co-factor" is meant any agent that is necessary for product. . .

DETD The **assay** also may contain other agents, such as stabilizing agents including, but not limited to, DTT and BSA. Such stabilizing agents. . .

DETD The necessary relative enzyme concentrations in the pathway **assay** can be predicted using the combination of kinetic parameters for each individual enzyme with a numeric model of the coupled. . .

DETD . . . pathway. This derived data and simulations are then used to construct initial conditions for the complete sequential mur enzyme pathway **assay**. This is done in such a way that each sequential enzyme will produce a nearly identical product flux throughout the **assay** such that inhibition of any enzyme will be reflected in the measured final product concentration. Identical product flux provides a. . . in which inhibition of any enzyme in the pathway is detected and that the inhibition results would be similar to **assaying** the enzyme individually. This type of analysis also allows for the validation of the coupled enzyme systems and a way. . .

DETD . . . 20 nM MurF; 10 .mu.M L-alanine; 500 nCi.sup.3 H-L-alanine; 100 .mu.M D-glutamate; 100 .mu.M meso-diaminopimelate; 100 .mu.M

D-alanine-D-alanine; 100 .mu.M **UDP**-N-acetyl muramic acid; 1 mM MgCl.sub.2 ; and 500 .mu.M ATP. These concentrations were set by variation of enzyme, substrate, co-factor,. . .

DETD Biologically active compounds are identified via pathway **assay** through a series of steps. Inhibitors are initially detected by a positive response, a change in the relative amount of. . . compared to a control set of data and points to the most probable inhibition target site(s). Second, the inhibitor is **reassayed** against each enzyme in the pathway individually in order to verify the inhibition target site(s).

DETD . . . IN/US radioflow detector. First, the HPLC profile is used to assess enzyme and substrate pool levels. Second, the inhibitor is **reassayed** against each enzyme in the pathway individually in order to verify the inhibition target site.

DETD Alternatively, the pathway **assay** of the present invention is be adopted to a format amenable for automated, high throughput mode. In one embodiment, this. . . MurD, MurE and MurF using radiolabeled D-alanine-D-alanine as a tracer. This format detects only the final product of the pathway, **UDP**-N-acetylmuramyl-pentapeptide. The principle of separation is absorption of the reaction product onto

AG1X8

resin (BioRad) followed by a washing step to remove unreacted radiolabeled D-alanine-D-alanine. Elution of the radiolabeled pathway product, **UDP**-N-acetylmuramyl-pentapeptide, may be accomplished using 1M salicylic acid. Inhibition of the pathway is detected as a reduction in the formation of radiolabeled **UDP** -N-acetylmuramyl-pentapeptide relative to a no inhibitor control. This **assay** can be automated in a 96 well format.

DETD The pathway **assay** in this high-throughput format contains the same components as that of the HPLC-based **assay**, except MurA, MurB, **UDP**-N-**acetylglucosamine** (replaces **UDP** -N-acetylmuramyl-L-alanine), PEP, NADPH and the radiolabeled tracer, D-alanine-D-alanine (replaces radiolabeled L-alanine). In a preferred embodiment, dithiothreitol (DTT) is added to exclude. . .

DETD The mur pathway **assay** contained 100 mM Bis-Tris Propane, pH 8.0; 10 .mu.M L-alanine (Sigma); 500 nCi .sup.3 H-L-ala (Amersham); 100 .mu.M D-glutamate (Sigma); 100 .mu.M meso-diaminopimelate (Sigma); 100 .mu.M D-alanine-D-alanine (Sigma); 100 .mu.M **UDP** N-acetyl muramic acid (enzymatically synthesized); 1 mM MgCl.sub.2 ; 500 .mu.M ATP. Equal volume of the solvent (i.e DMSO) was. . .

DETD The Murein Pathway **assay**

DETD The mur pathway **assay** contained 100 mM Bis-Tris Propane, pH 8.0; 10 .mu.M L-alanine (Sigma); 500 nCi.sup.3 H-L-ala (Amersham); 100 .mu.M D-glutamate (Sigma); 100 .mu.M meso-diaminopimelate (Sigma); 100 .mu.M D-alanine-D-alanine (Sigma); 100 .mu.M **UDP** N-acetyl muramic acid (enzymatically synthesized); 1 mM MgCl.sub.2; 500 .mu.M ATP. An inhibitor, compound 3, Tanner, et al., "Phosphinate Inhibitors of the D-Glutamic Acid-Adding Enzyme of **Peptidoglycan** Biosynthesis," 61 J. Org. Chem. 1756-1760 (1996), was added to a final concentration of 100 .mu.M. Equal volume of the. . .

DETD The mur pathway **assay** in this high-throughput format contained 100 mM Bis-Tris Propane, pH 8.0; 100 .mu.M L-alanine (Sigma); 100 .mu.M D-glutamate (Sigma); 100 .mu.M meso-diaminopimelate (Sigma); 10 .mu.M D-alanine-D-alanine (Sigma); 500 nCi.sup.3 H-D-alanine-D-alanine or .sup.14 C-D-alanine-D-alanine (ARC, Inc.); 12 .mu.M **UDP**-N-**acetyl -glucosamine** (Sigma); 25 .mu.M NADPH (Sigma); 12 .mu.M PEP (Sigma); 500 .mu.M DTT (Sigma); 25 mM (NH.sub.4).sub.2 SO.sub.4 ; 5 mM. . .

DETD The mur pathway **assay** in this high-throughput format contained 100 mM Bis-Tris Propane, pH 8.0; 100 .mu.M L-alanine (Sigma); 100 .mu.M D-glutamate (Sigma); 100 .mu.M meso-diaminopimelate (Sigma); 10 .mu.M D-alanine-D-alanine (Sigma); 500 nCi.sup.3 H-D-alanine-D-alanine or .sup.14 C-D-alanine-D-alanine (ARC); 12 .mu.M **UDP**-N-**acetyl-glucosamine** (Sigma); an inhibitor, fosfomycin (Sigma) was added to a final concentration of 12 .mu.M; 25 .mu.M NADPH (Sigma); 12 .mu.M. . .

DETD The mur pathway **assay** may be arranged in preincubation mode. In this format the **assay** contained 100 mM Bis-Tris Propane, pH 8.0; 100 .mu.M L-alanine (Sigma); 100 .mu.M D-glutamate (Sigma); 100 1.mu.M meso-diaminopimelate (Sigma); 10 .mu.M D-alanine-D-alanine (Sigma); 500 nCi.sup.3 H-D-alanine-D-alanine or .sup.14 C-D-alanine-D-alanine (ARC); 12 .mu.M **UDP**-N-**acetyl-glucosamine** (Sigma); an inhibitor, fosfomycin (Sigma) was added to a final concentration of 12 .mu.M; 25 .mu.M NADPH (Sigma); 500 .mu.M.

. .

DETD Synthesis of **UDP**-N-acetylmuramic acid (UDPMurNAc)

DETD UDPMurNAc was synthesized by a coupled MurA, MurB reaction consisting of

0.5 g (0.75 mmol) **UDP**-N-**acetylglucosamine** (Sigma), 0.38 g (0.85 mmol) phosphoenoyl-pyruvate tricyclohexylammonium salt (Sigma); 0.7 g (0.75 mmol) .beta.-NADPH (Sigma) and 50 mM Bis-Tris-Propane pH. . . UDPMurNAc was purified by a modification of the published procedure found in Jin, et al, "Structural Studies of Escherichia coli **UDP**-N-Acetylmuramate:L-Alanine Ligase," 35 Biochemistry, 1423-1431, (1996). The modified procedure was as follows. The reaction was ultrafiltrated using an Amicon equipped with. . . mM. UDPMurNAc eluted at 150 mM concentration. Fractions were pooled and lyopholized to yielded a white solid. For the pathway **assay**, the solid was brought up to a concentration of typically 10 mM.

CLM What is claimed is:

13. The kit **assay** according to claim 12 wherein the first enzyme is suitable for changing the substrate for the first enzyme into a. . .

16. The kit according to claim 15 wherein the substrate for the first enzyme is uridyl-5'-diphosphate-N-**acetyl-glucosamine**; the co-substrate for the first enzyme is phosphoenolpyruvate; the substrate for the second enzyme is uridyl-5'-diphosphate-N-acetyl-enolpyruvylglucosamine; the co-substrates for the. . .

36. The method according to claim 35 wherein the substrate for the first enzyme is uridyl-5'-diphosphate-N-**acetyl-glucosamine**; the co-substrate for the first enzyme is phosphoenolpyruvate; the substrate for the second enzyme is uridyl-5'-diphosphate-N-acetyl-enolpyruvylglucosamine; the co-substrates for the. . .

L19 ANSWER 10 OF 17 USPATFULL

AB The invention provides isolated nucleic acid compounds encoding the murE stem peptide biosynthetic gene of Streptococcus pneuinoniae. Also provided are vectors and transformed heterologous host cells for expressing the murE enzyme product and a method for identifying compounds that inhibit stem peptide biosynthesis.

TI **Peptidoglycan** biosynthetic gene murE from Streptococcus pneumoniae

SUMM The bacterial cell wall structure comprises a **peptidoglycan** layer which provides mechanical rigidity for the bacterium. This segment of the cell wall is composed of a sugar backbone (alternating residues of N-**acetylglucosamine** and N-acetylmuramic acid) attached to a pentapeptide (also referred to as "stem peptide," or "Park nucleotide") containing alternating D and L amino acid residues. The nascent **peptidoglycan** layer is stabilized by an enzymatic step which crosslinks adjacent pentapeptide moieties. Without this crosslinking step the **peptidoglycan** structure is severely weakened and susceptible to degradation. Indeed, it is this crosslinking step that has been a frequently targeted. . .

SUMM . . . the UDPGlcNAc enolpyruvyl transferase and NADH-dependent reductase, UDPGlcNAc is converted to UDPMurNAc. In five subsequent steps, catalyzed by N-acetylmuramate:L-alanine ligase; **UDP**-N-acetyl-muramoyl-L-alanine:D-glutamate ligase; **UDP**-N-acetyl-muramoyl-L-alanyl-D-glutamate:lysine ligase; **UDP**

-N-acetylmuramoyl-L-alanyl-D-glutamyl-L-lysine:D-alanyl-D-alanine ligase; and D-alanyl-D-alanine synthetase, the final product, UDPMurNAc-L-Ala-D-Glu-L-lysine-D-Ala-D-Ala, is produced in Streptococcus pneumoniae.

SUMM . . . which target this pathway, have been developed. For example, D-cycloserine, inhibits the alanine racemase and the D-alanine-D-alanine synthetase; phosphonomycin inhibits **UDP-GlcNac** conversion to **UDP-GlcNac**-enolpyruvate; and Ala-phosphonine inhibits the addition of L-Alanine in the formation of **UDP-MurNac**-L-Ala.

DETD The murE gene of Streptococcus pneumoniae encodes an **enzyme** involved in stem peptide biosynthesis. The stem peptide pathway is necessary for the synthesis of the **peptidoglycan** layer which is part of the **bacterial** cell wall. There are at least 10 steps involved in stem peptide biosynthesis. The murE gene encodes uridine-diphosphate-N-acetyulmuramoyl-L-alanyl-D-glutamate:L-lysine ligase (SEQ. . .

DETD . . . transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily **assayable** or selectable markers (e.g., antibiotic resistance markers, metabolic markers, or the like), and the number of copies of the gene. . .

DETD Biochemical **Assay** for Inhibitors of Streptococcus pneumoniae MurE Enzyme Product

DETD The activity of the **enzyme** encoded by murE was **assayed** by monitoring the appearance of the **enzyme** product, **UDP-MurNAc**-L-Ala-D-Glu-L-Lys, using high-pressure liquid chromatography detection (HPLC). The **enzyme** reaction consisted of 0.1M Tris/HCl pH 8.6, 0.1M MgCl.sub.2, 5 mM ATP, 50 .mu.M **UDP-MurNAc**-L-Ala-D-Glu, 0.1 mM Lysine and **enzyme** in a final volume of 50 .mu.l. Substrate **UDP-MurNAc**-L-Ala-D-Glu was purified as described in B. Flouret et al., Reverse-phase high-pressure liquid chromatography of uridine diphosphate N-Acetylmuramyl peptide precursors of **bacterial** cell wall **peptidoglycan**. Anal. Biochem. 114, 59-63 (1981). The mixture was incubated for 30 min. at 37.degree. C., and the reaction terminated with. . . extracted in the cold by trichloroacetic acid and purified by gel filtration on fine SEPHADEX G-25. Under these conditions the **UDP-MurNac** derivatives are eluted with water in a volume slightly larger than the exclusion volume of the column. Separation and further purification of **UDP-MurNAc** derivatives were carried out by ion-exchange chromatography on Dowex AG1.times.2 (200-400 mesh) according to the method of Park & Chatterjee,. . .

L18 ANSWER 5 OF 21 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000252680 MEDLINE
DOCUMENT NUMBER: 20252680
TITLE: **Assay** for identification of inhibitors for bacterial MraY translocase or MurG transferase.
AUTHOR: Branstrom A A; Midha S; Longley C B; Han K; Baizman E R; Axelrod H R
CORPORATE SOURCE: Department of Biological Research, IRL, Inc., Cranbury, New Jersey, 08512, USA.. art@irl.incara.com
SOURCE: ANALYTICAL BIOCHEMISTRY, (2000 May 1) 280 (2) 315-9. Journal code: 4NK. ISSN: 0003-2697.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200008
ENTRY WEEK: 20000803

=>

=> d l18 ab kwic 5

L18 ANSWER 5 OF 21 MEDLINE DUPLICATE 1
AB Bacterial **peptidoglycan** synthesis is a well-characterized system for targeting new antimicrobial drugs. Formation of the **peptidoglycan** precursors Lipid I and Lipid II is catalyzed by the gene products of mraY and murG, which are involved in the first and second steps of the lipid cycle reactions, respectively. Here we describe the development of an **assay** specific for identifying inhibitors of MraY or MurG, based on the detection of radiolabeled [(14)C]**GlcNAc** incorporated into Lipid II. **Assay** specificity is achieved with the biotin tagging of the Lipid I precursor **UDP-MurNAc** -pentapeptide. This allows for the separation and identification of lipid products produced by the enzymatic activity of the MraY and MurG proteins, and thus identification of specific inhibitors. Copyright 2000 Academic Press.
TI **Assay** for identification of inhibitors for bacterial MraY translocase or MurG transferase.
AB Bacterial **peptidoglycan** synthesis is a well-characterized system for targeting new antimicrobial drugs. Formation of the **peptidoglycan** precursors Lipid I and Lipid II is catalyzed by the gene products of mraY and murG, which are involved in the first and second steps of the lipid cycle reactions, respectively. Here we describe the development of an **assay** specific for identifying inhibitors of MraY or MurG, based on the detection of radiolabeled [(14)C]**GlcNAc** incorporated into Lipid II. **Assay** specificity is achieved with the biotin tagging of the Lipid I precursor **UDP-MurNAc** -pentapeptide. This allows for the separation and identification of lipid products produced by the enzymatic activity of the MraY and MurG. . .
CT . . . Wall: CH, chemistry
*Enzyme Inhibitors: AN, analysis
Escherichia coli
Lipids: ME, metabolism

*N-Acetylglucosaminyltransferases: AI, antagonists & inhibitors
N-Acetylglucosaminyltransferases: ME, metabolism
Peptidoglycan: ME, metabolism
Uridine Diphosphate N-Acetylmuramic Acid: AA, analogs & derivatives
Uridine Diphosphate N-Acetylmuramic Acid: IP, isolation & purification
Uridine. . .

RN **16124-22-4 (UDP-N-acetylmuramic acid pentapeptide)**

CN EC 2.4.1.- (N-Acetylglucosaminyltransferases); EC 2.4.1.- (**UDP**
-N-acetylglucosamine-N-acetylmuramyl-
(pentapeptide)pyrophosphoryl-undecaprenol N-**acetylglucosamine**
transferase); 0 (mraY protein); 0 (Bacterial Proteins); 0 (Enzyme
Inhibitors); 0 (Lipids); 0 (**Peptidoglycan**); 0 (Uridine
Diphosphate N-Acetylmuramic Acid)

L18 ANSWER 6 OF 21 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000141264 MEDLINE
DOCUMENT NUMBER: 20141264
TITLE: Chlorobiphenyl-desleucyl-vancomycin inhibits the transglycosylation process required for **peptidoglycan** synthesis in bacteria in the absence of dipeptide binding.
AUTHOR: Goldman R C; Baizman E R; Longley C B; Branstrom A A
CORPORATE SOURCE: Incara Research Laboratories, 8 Cedar Brook Drive, Cranbury, NJ 08512, USA.. rgoldman@irl.incara.com
SOURCE: FEMS MICROBIOLOGY LETTERS, (2000 Feb 15) 183 (2) 209-14. Journal code: FML. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY WEEK: 20000602

AB Novel glycopeptide analogs are known that have activity on vancomycin resistant enterococci despite the fact that the primary site for drug interaction, D-ala-D-ala, is replaced with D-ala-D-lactate. The mechanism of action of these compounds may involve dimerization and/or membrane binding, thus enhancing interaction with D-ala-D-lactate, or a direct interaction with the transglycosylase enzymes involved in **peptidoglycan** polymerization. We evaluated the ability of vancomycin (V), desleucyl-vancomycin (desleucyl-V), chlorobiphenyl-vancomycin (CBP-V), and chlorobiphenyl-desleucyl-vancomycin (CBP-desleucyl-V) to inhibit (a) **peptidoglycan** synthesis in vitro using **UDP**-muramyl-pentapeptide and **UDP**-muramyl-tetrapeptide substrates and (b) growth and **peptidoglycan** synthesis in vancomycin resistant enterococci. Compared to V or CBP-V, CBP-desleucyl-V retained equivalent potency in these **assays**, whereas desleucyl-V was inactive. In addition, CBP-desleucyl-V caused accumulation of N-**acetylglucosamine**-beta-1, 4-**MurNAc**-pentapeptide-pyrophosphoryl-undecaprenol (lipid II). These data show that CBP-desleucyl-V inhibits **peptidoglycan** synthesis at the transglycosylation stage in the absence of binding to dipeptide.

TI Chlorobiphenyl-desleucyl-vancomycin inhibits the transglycosylation process required for **peptidoglycan** synthesis in bacteria in the absence of dipeptide binding.

AB . . . involve dimerization and/or membrane binding, thus enhancing interaction with D-ala-D-lactate, or a direct interaction with the transglycosylase enzymes involved in **peptidoglycan** polymerization. We evaluated the ability of vancomycin (V), desleucyl-vancomycin (desleucyl-V), chlorobiphenyl-vancomycin (CBP-V), and chlorobiphenyl-desleucyl-vancomycin (CBP-desleucyl-V) to inhibit (a) **peptidoglycan** synthesis in vitro using **UDP**-muramyl-pentapeptide and **UDP**-muramyl-tetrapeptide substrates and (b) growth and **peptidoglycan** synthesis in vancomycin resistant enterococci. Compared to V or CBP-V, CBP-desleucyl-V retained equivalent potency in these **assays**, whereas desleucyl-V was inactive. In addition, CBP-desleucyl-V caused accumulation of N-**acetylglucosamine**-beta-1, 4-**MurNAc**-pentapeptide-pyrophosphoryl-undecaprenol (lipid II). These data show that CBP-desleucyl-V inhibits **peptidoglycan** synthesis at the transglycosylation stage in the absence of binding to dipeptide.

CT *Antibiotics, Glycopeptide: PD, pharmacology
*Bacteria: DE, drug effects
*Bacteria: ME, metabolism
Dipeptides: ME, metabolism
Glycosylation
***Peptidoglycan*: BI, biosynthesis**
*Vancomycin: AA, analogs & derivatives
Vancomycin: PD, pharmacology

CN 0 (chlorobiphenyl-desleucyl-vancomycin); 0 (Antibiotics, Glycopeptide); 0 (Dipeptides); 0 (**Peptidoglycan**)

L18 ANSWER 16 OF 21 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:131550 CAPLUS
DOCUMENT NUMBER: 128:150907
TITLE: Substrate synthesis and activity **assay** for MurG
AUTHOR(S): Men, Hongbin; Park, Peter; Ge, Min; Walker, Suzanne
CORPORATE SOURCE: Department of Chemistry, Princeton University, Princeton, NJ, 08544, USA
SOURCE: J. Am. Chem. Soc. (1998), 120(10), 2484-2485
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Some of the best antibiotics function by interfering with the biosynthesis of the **peptidoglycan** polymer that surrounds bacterial cells. With the emergence of bacterial pathogens that are resistant to common antibiotics it has become imperative to learn more about the enzymes involved in **peptidoglycan** biosynthesis. Unfortunately, many of the enzymes have proven exceedingly difficult to study. One such enzyme is MurG, a cytoplasmic membrane-assocd. enzyme that transfers **UDP**-N-**acetylglucosamine** to the C4 hydroxyl of a lipid-linked muramic acid deriv. MurG may be termed **UDP**-N-**acetylglucosamine**:N-acetylmuramyl(pentapeptide) pyrophosphoryl-undecaprenol N-**acetylglucosamine** transferase. Because the lipid-linked substrate for MurG is extremely difficult to isolate, no direct **assays** for MurG activity have been developed. Consequently, it has not been possible to purify MurG or to det. the minimal functional length; nor has it been possible to carry out any detailed mechanistic studies, or to det. the substrate requirements. Here we report the synthesis of a substrate for MurG and show that it can be used in a direct and rapid **assay** for enzyme activity. This substrate and activity **assay** should make possible detailed mechanistic and structural analyses of the wholly or partially purified MurG enzyme.

TI Substrate synthesis and activity **assay** for MurG

AB Some of the best antibiotics function by interfering with the biosynthesis of the **peptidoglycan** polymer that surrounds bacterial cells. With the emergence of bacterial pathogens that are resistant to common antibiotics it has become imperative to learn more about the enzymes involved in **peptidoglycan** biosynthesis. Unfortunately, many of the enzymes have proven exceedingly difficult to study. One such enzyme is MurG, a cytoplasmic membrane-assocd. enzyme that transfers **UDP**-N-**acetylglucosamine** to the C4 hydroxyl of a lipid-linked muramic acid deriv. MurG may be termed **UDP**-N-**acetylglucosamine**:N-acetylmuramyl(pentapeptide) pyrophosphoryl-undecaprenol N-**acetylglucosamine** transferase. Because the lipid-linked substrate for MurG is extremely difficult to isolate, no direct **assays** for MurG activity have been developed. Consequently, it has not been possible to purify MurG or to det. the minimal functional length; nor has it been possible to carry out any detailed mechanistic studies, or to det. the substrate requirements. Here we report the synthesis of a substrate for MurG and show that it can be used in a direct and rapid **assay** for enzyme activity. This substrate and activity **assay** should make possible detailed

mechanistic and structural analyses of the wholly or partially purified MurG enzyme.

ST **UDP acetylglucosamine acetylmuramylpentapeptide**** * **pyrophosphorylundecaprenol** *****acetylglucosamine** transferase; enzyme MurG **assay** substrate

IT 202831-89-8P
RL: ARG (Analytical reagent use); SPN (Synthetic preparation); ANST (Analytical study); PREP (Preparation); USES (Uses)
(MurG substrate; substrate synthesis and activity **assay** for enzyme MurG (uridine diphosphoacetylglucosamine-acetylmuramoylpentapeptide pyrophospholipid acetylglucosaminyltransferase))

IT 18867-73-7 73089-68-6 202831-85-4 202831-86-5
RL: RCT (Reactant)
(in MurG substrate prepn.; substrate synthesis and activity **assay** for enzyme MurG (uridine diphosphoacetylglucosamine-acetylmuramoylpentapeptide pyrophospholipid acetylglucosaminyltransferase))

IT 202831-88-7P
RL: SPN (Synthetic preparation); PREP (Preparation)
(in MurG substrate prepn.; substrate synthesis and activity **assay** for enzyme MurG (uridine diphosphoacetylglucosamine-acetylmuramoylpentapeptide pyrophospholipid acetylglucosaminyltransferase))

IT 60976-26-3
RL: ANT (Analyte); BAC (Biological activity or effector, except adverse); ANST (Analytical study); BIOL (Biological study)
(substrate synthesis and activity **assay** for enzyme MurG (uridine diphosphoacetylglucosamine-acetylmuramoylpentapeptide pyrophospholipid acetylglucosaminyltransferase))

L18 ANSWER 18 OF 21 USPATFULL

ACCESSION NUMBER:	97:99148 USPATFULL	
TITLE:	Murd protein method and kit for identification of inhibitors	
INVENTOR(S):	Hoskins, Jo Ann, Indianapolis, IN, United States Peery, Robert Brown, Brownsburg, IN, United States Skatrud, Paul Luther, Indianapolis, IN, United States Wu, Chyun-Yeh Earnest, Indianapolis, IN, United States	
PATENT ASSIGNEE(S):	Eli Lilly and Company, Indianapolis, IN, United States (U.S. corporation)	
	NUMBER	DATE
PATENT INFORMATION:	US 5681694	19971028
APPLICATION INFO.:	US 1996-665435	19960618 (8)
DOCUMENT TYPE:	Utility	
PRIMARY EXAMINER:	Wax, Robert A.	
ASSISTANT EXAMINER:	Stole, Einar	
LEGAL REPRESENTATIVE:	Webster, Thomas D.; Boone, David E.	
NUMBER OF CLAIMS:	5	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	1 Drawing Figure(s); 1 Drawing Page(s)	
LINE COUNT:	747	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides isolated nucleic acid compounds encoding the murD stem peptide biosynthetic gene of Streptococcus pneumoniae. Also provided are vectors and transformed heterologous host cells for expressing the MurD enzyme product and a method for identifying compounds that inhibit stem peptide biosynthesis.

SUMM The bacterial cell wall structure contains a **peptidoglycan** layer which provides mechanical rigidity for the bacterium. This segment of the cell wall is composed of a sugar backbone (alternating residues of N-**acetylglucosamine** and N-acetylmuramic acid) attached to a

pentapeptide (also referred to as "stem peptide," or "Park nucleotide") containing alternating D and L amino acid residues. The nascent **peptidoglycan** layer is stabilized by an enzymatic step which crosslinks adjacent pentapeptide moieties. Without this crosslinking step the **peptidoglycan** structure is severely weakened and susceptible to degradation. Indeed, it is this crosslinking step that has been a frequently targeted. . .

SUMM . . . catalyzed by the UDPGlcNAc enolpyruvyl transferase and NADH-dependent reductase, UDPGlcNAc is converted to UDPMurNAc. In five subsequent steps, catalyzed by **UDP**-N-acetylmuramate:L-alanine ligase; **UDP**-N-acetyl-muramyl-L-alanine:D-glutamate ligase; **UDP**-N-acetyl-muramyl-L-alanyl-D-isoglutamate:L-lysine ligase; **UDP**-N-acetylmuramyl-L-alanyl-D-isoglutamyl-L-lysine:D-alanyl-D-alanine ligase; and D-alanyl-D-alanine synthetase, the final product, UDPMurNAc-L-Ala-D-isoGlu-L-lysine-D-Ala-D-Ala, is produced in Streptococcus pneumoniae.

SUMM . . . target this pathway, have been developed. For example, D-cycloserine inhibits alanine racemase and D-alanine-D-alanine synthetase; phosphonomycin inhibits the conversion of **UDP-GlcNAc** to **UDP-GlcNac**-enolpyruvate; and Ala-phosphonine inhibits the formation of **UDP-MurNac** -L-Ala.

DETD . . . pneumoniae encodes an enzyme involved in stem peptide biosynthesis. The stem peptide pathway is necessary for the synthesis of the **peptidoglycan** layer which is part of the bacterial cell wall. There are at least 10 steps involved in stem peptide biosynthesis.. . .

DETD . . . transformation (e.g., stable transformation as an extrachromosomal element, or integration into the host chromosome), the presence or absence of readily **assayable** or selectable markers (e.g., antibiotic resistance markers, metabolic markers, or the like), and the number of copies of the gene. . .

DETD Biochemical **Assay** for Inhibitors of Streptococcus pneumoniae MurD Enzyme Product

DETD The activity of the MurD enzyme was **assayed** by monitoring the appearance of the enzyme product, **UDP-MurNAc** -L-Ala-D-isoGlu, using high-pressure liquid chromatography detection (HPLC). The enzyme reaction consisted of 0.1M Tris/HCl pH 8.6, 20 mM MgCl.sub.2, 5 mM ATP, 100 .mu.M **UDP-MurNAc**-L-Ala, 50 .mu.M D-glutamic acid, and enzyme in a final volume of 50 .mu.l. Substrate **UDP-MurNAc**-L-Ala was purified as described in B. Flouret et al., Reverse-phase high-pressure liquid chromatography of uridine diphosphate N-Acetylmuramyl peptide precursors of bacterial cell wall **peptidoglycan**, Anal. Biochem. 114, 59-63 (1981). The mixture was incubated for 30 min. at 37.degree. C., and the reaction terminated with. . . nucleotide precursors were extracted in the cold by trichloroacetic acid and purified by gel filtration on fine SEPHADEX G-25. The **UDP-MurNac** derivatives were eluted with water in a volume slightly larger than the exclusion volume of the column. Separation and further purification of **UDP-MurNAc** derivatives were carried out by ion-exchange chromatography on Dowex AG1.times.2 (200-400 mesh) according to the method of Park & Chatterjee,. . .

CLM What is claimed is:
3. A method, as in claim 2 wherein the substrate of step (a) (ii) comprises **UDP-MurNAc**-L-Ala.

L18 ANSWER 19 OF 21 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 91310568 MEDLINE
DOCUMENT NUMBER: 91310568
TITLE: The murG gene of Escherichia coli codes for the **UDP** -N-**acetylglucosamine**: N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-

acetylglucosamine transferase involved in the membrane steps of **peptidoglycan** synthesis.

AUTHOR: Mengin-Lecreulx D; Texier L; Rousseau M; van Heijenoort J
CORPORATE SOURCE: Laboratoire de Biochimie Moleculaire et Cellulaire, URA 1131, Centre National de la Recherche Scientifique, Universite Paris-Sud, Orsay, France..

SOURCE: JOURNAL OF BACTERIOLOGY, (1991 Aug) 173 (15) 4625-36.
Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199110

AB Physiological properties of the murG gene product of Escherichia coli were investigated. The inactivation of the murG gene rapidly inhibits **peptidoglycan** synthesis in exponentially growing cells. As a result, various alterations of cell shape are observed, and cell lysis finally occurs when the **peptidoglycan** content is 40% lower than that of normally growing cells. Analysis of the pools of **peptidoglycan** precursors reveals the concomitant accumulation of **UDP**-N-**acetylglucosamine** (**UDP-GlcNAc**) and **UDP**-N-acetylmuramyl-pentapeptide (**UDP-MurNAc**-pentapeptide) and, to a lesser extent, that of undecaprenyl-pyrophosphoryl-**MurNAc**-pentapeptide (lipid intermediate I), indicating that inhibition of **peptidoglycan** synthesis occurs after formation of the cytoplasmic precursors. The relative depletion of the second lipid intermediate, undecaprenyl-pyrophosphoryl-**MurNAc**-(pentapeptide)**GlcNAc**, shows that inactivation of the murG gene product does not prevent the formation of lipid intermediate I but inhibits the next reaction in which **GlcNAc** is transferred to lipid intermediate I. In vitro **assays** for phospho-**MurNAc**-pentapeptide translocase and N-acetylglucosaminyl transferase activities finally confirm the identification of the murG gene product as the transferase that catalyzes the conversion of lipid intermediate I to lipid intermediate II in the **peptidoglycan** synthesis pathway. Plasmids allowing for a high overproduction of the transferase and the determination of its N-terminal amino acid sequence were constructed. In cell fractionation experiments, the transferase is essentially associated with membranes when it is recovered.

TI The murG gene of Escherichia coli codes for the **UDP**-N-**acetylglucosamine**: N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-**acetylglucosamine** transferase involved in the membrane steps of **peptidoglycan** synthesis.

AB Physiological properties of the murG gene product of Escherichia coli were investigated. The inactivation of the murG gene rapidly inhibits **peptidoglycan** synthesis in exponentially growing cells. As a result, various alterations of cell shape are observed, and cell lysis finally occurs when the **peptidoglycan** content is 40% lower than that of normally growing cells. Analysis of the pools of **peptidoglycan** precursors reveals the concomitant accumulation of **UDP**-N-**acetylglucosamine** (**UDP-GlcNAc**) and **UDP**-N-acetylmuramyl-pentapeptide (**UDP-MurNAc**-pentapeptide) and, to a lesser extent, that of undecaprenyl-pyrophosphoryl-**MurNAc**-pentapeptide (lipid intermediate I), indicating that inhibition of **peptidoglycan** synthesis occurs after formation of the cytoplasmic precursors. The relative depletion of the second lipid intermediate, undecaprenyl-pyrophosphoryl-**MurNAc**-(pentapeptide)**GlcNAc**, shows that inactivation of the murG gene product does not prevent the formation of lipid intermediate I but inhibits the next reaction in which **GlcNAc** is transferred to lipid intermediate I. In vitro **assays** for phospho-**MurNAc**-pentapeptide translocase and N-acetylglucosaminyl transferase activities finally confirm the

identification of the murG gene product as the transferase that catalyzes the conversion of lipid intermediate I to lipid intermediate II in the **peptidoglycan** synthesis pathway. Plasmids allowing for a high overproduction of the transferase and the determination of its N-terminal amino acid sequence. . .

CT . . .

Glucosyltransferases: BI, biosynthesis
*Glucosyltransferases: GE, genetics
Glucosyltransferases: IP, isolation & purification
Glycolipids: ME, metabolism
Molecular Sequence Data
Molecular Weight
Mutation
***Peptidoglycan: BI, biosynthesis**
Peptidoglycan: GE, genetics
Phenotype
Phosphotransferases: GE, genetics
Plasmids
Subcellular Fractions: CH, chemistry

RN **109138-08-1 (N-acetylglucosamine-pyrophosphorylundecaprenol)**

CN EC 2.4.1.- (Glucosyltransferases); EC 2.4.1.- (**UDP-N-acetylglucosamine**-N-acetylmuramyl-(pentapeptide)pyrophosphoryl-undecaprenol N-**acetylglucosamine** transferase); EC 2.7 (Phosphotransferases); EC 2.7.8.13 (phospho-N-acetylmuramoyl pentapeptide transferase); 0 (Bacterial Outer Membrane Proteins); 0 (Glycolipids); 0 (Plasmids)

L17 ANSWER 5 OF 25 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:281601 CAPLUS
DOCUMENT NUMBER: 133:101178
TITLE: Assay for Identification of Inhibitors for Bacterial MraY Translocase or MurG Transferase
AUTHOR(S): Branstrom, Arthur A.; Midha, Sunita; Longley, Clifford B.; Han, Kiho; Baizman, Eugene R.; Axelrod, Helena R.
CORPORATE SOURCE: Department of Biological Research, IRL, Inc., Cranbury, NJ, 08512, USA
SOURCE: Anal. Biochem. (2000), 280(2), 315-319
CODEN: ANBCA2; ISSN: 0003-2697
PUBLISHER: Academic Press
DOCUMENT TYPE: Journal
LANGUAGE: English
REFERENCE COUNT: 14
REFERENCE(S):
(2) Brandish, P; Antimicrob Agents Chemother 1996, V40, P1640 CAPLUS
(5) Ikeda, M; J Bacteriol 1991, V173, P1021 CAPLUS
(7) Men, H; J Am Chem Soc 1998, V120, P2484 CAPLUS
(8) Mengin-Lecreulx, D; J Bacteriol 1991, V173, P4625 CAPLUS
(9) Mirelman, D; Biochem Biophys Res Commun 1972, V46, P1909 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d l17 ab kwic 5

L17 ANSWER 5 OF 25 CAPLUS COPYRIGHT 2000 ACS
AB Bacterial **peptidoglycan** synthesis is a well-characterized system for targeting new antimicrobial drugs. Formation of the **peptidoglycan** precursors Lipid I and Lipid II is catalyzed by the gene products of mraY and murG, which are involved in the first and second steps of the lipid cycle reactions, resp. Here we describe the development of an assay specific for identifying inhibitors of MraY or MurG, based on the detection of radiolabeled [14C]**GlcNAc** incorporated into Lipid II. Assay specificity is achieved with the biotin tagging of the Lipid I precursor **UDP-MurNAc** -pentapeptide. This allows for the sepn. and identification of lipid products produced by the enzymic activity of the MraY and MurG proteins, and thus identification of specific inhibitors. (c) 2000 Academic Press.
AB Bacterial **peptidoglycan** synthesis is a well-characterized system for targeting new antimicrobial drugs. Formation of the **peptidoglycan** precursors Lipid I and Lipid II is catalyzed by the gene products of mraY and murG, which are involved in. . . describe the development of an assay specific for identifying inhibitors of MraY or MurG, based on the detection of radiolabeled [14C]**GlcNAc** incorporated into Lipid II. Assay specificity is achieved with the biotin tagging of the Lipid I precursor **UDP-MurNAc** -pentapeptide. This allows for the sepn. and identification of lipid products produced by the enzymic activity of the MraY and MurG. . .
IT Glycopeptides

RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(muramic acid-contg., **UDP**-Mur-NAc-pentapeptide, biotinylated; assay is based on transfer of N-acetyl-D-glucosamine from uridine-diphosphate)

IT 60976-26-3, Gene MurG **enzyme**
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(MurG; assay for identification of inhibitors of **bacterial** MraY translocase or MurG transferase)

IT 7512-17-6, N-**Acetyl-glucosamine**
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(assay is based on transfer of N-acetyl-D-glucosamine from uridine-diphosphate)

L17 ANSWER 15 OF 25 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:131550 CAPLUS
DOCUMENT NUMBER: 128:150907
TITLE: Substrate synthesis and activity assay for MurG
AUTHOR(S): Men, Hongbin; Park, Peter; Ge, Min; Walker, Suzanne
CORPORATE SOURCE: Department of Chemistry, Princeton University, Princeton, NJ, 08544, USA
SOURCE: J. Am. Chem. Soc. (1998), 120(10), 2484-2485
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

=> d l17 ab kwic 15

L17 ANSWER 15 OF 25 CAPLUS COPYRIGHT 2000 ACS
AB Some of the best antibiotics function by interfering with the biosynthesis of the **peptidoglycan** polymer that surrounds **bacterial** cells. With the emergence of **bacterial** pathogens that are resistant to common antibiotics it has become imperative to learn more about the **enzymes** involved in **peptidoglycan** biosynthesis. Unfortunately, many of the **enzymes** have proven exceedingly difficult to study. One such **enzyme** is MurG, a cytoplasmic membrane-assocd. **enzyme** that transfers **UDP**-N-**acetylglucosamine** to the C4 hydroxyl of a lipid-linked muramic acid deriv. MurG may be termed **UDP**-N-**acetylglucosamine**:N-acetylmuramyl(pentapeptide) pyrophosphoryl-undecaprenol N-**acetylglucosamine** transferase. Because the lipid-linked substrate for MurG is extremely difficult to isolate, no direct assays for MurG activity have been developed. Consequently, it has not been possible to purify MurG or to det. the minimal functional length; nor has it been possible to carry out any detailed mechanistic studies, or to det. the substrate requirements. Here we report the synthesis of a substrate for MurG and show that it can be used in a direct and rapid assay for **enzyme** activity. This substrate and activity assay should make possible detailed mechanistic and structural analyses of the wholly or partially purified MurG **enzyme**.
AB Some of the best antibiotics function by interfering with the biosynthesis of the **peptidoglycan** polymer that surrounds **bacterial** cells. With the emergence of **bacterial** pathogens that are resistant to common antibiotics it has become imperative to learn more about the **enzymes** involved in **peptidoglycan** biosynthesis. Unfortunately, many of the **enzymes** have proven exceedingly difficult to study. One such **enzyme** is MurG, a cytoplasmic membrane-assocd. **enzyme** that transfers **UDP**-N-**acetylglucosamine** to the C4 hydroxyl of a lipid-linked muramic acid deriv. MurG may be termed **UDP**-N-**acetylglucosamine**:N-acetylmuramyl(pentapeptide) pyrophosphoryl-undecaprenol N-**acetylglucosamine** transferase. Because the lipid-linked substrate for MurG is extremely difficult to isolate, no direct assays for MurG activity have been. . . synthesis of

a substrate for MurG and show that it can be used in a direct and rapid assay for **enzyme** activity. This substrate and activity assay should make possible detailed mechanistic and structural analyses of the wholly or partially purified MurG **enzyme**.

ST **UDP acetylglucosamine acetylmuramylpentapeptide****
* **pyrophosphorylundecaprenol** ***acetylglucosamine** transferase; enzyme MurG assay substrate

L17 ANSWER 18 OF 25 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1997:489989 CAPLUS
TITLE: Inhibitors of the **bacterial** cell wall biosynthesis **enzyme** Mur D.
AUTHOR(S): Gegnas, Laura D.; Waddell, Sherman T.; Chabin, Renee M.; Reddy, Sreelatha; Wong, Kenny K.
CORPORATE SOURCE: Merck Research Laboratories, Rahway, NJ, 07065, USA
SOURCE: Book of Abstracts, 214th ACS National Meeting, Las Vegas, NV, September 7-11 (1997), MEDI-232. American Chemical Society: Washington, D. C.
CODEN: 64RNAO
DOCUMENT TYPE: Conference; Meeting Abstract
LANGUAGE: English
AB The **bacterial** cell wall **peptidoglycan** layer consists of alternating N-acetyl muramic acid (**MurNAc**) and N-**acetyl glucosamine** units that are crosslinked through pentapeptide chains. The disruption of this structure leads to cell lysis; **peptidoglycan** biosynthesis is therefore an essential pathway and an important target for antibiotics research. The **enzyme** MurD catalyzes the addn. of D-glutamate to uridine diphosphate(**UDP**)-**MurNAc**-L-alanine in the biosynthesis of the **peptidoglycan** precursor **UDP-MurNAc** -pentapeptide. We have designed a mechanism-based phosphinate inhibitor of MurD (1). The synthesis and inhibitory activity of compd. 1 and its precursors will be discussed.
TI Inhibitors of the **bacterial** cell wall biosynthesis **enzyme** Mur D.
AB The **bacterial** cell wall **peptidoglycan** layer consists of alternating N-acetyl muramic acid (**MurNAc**) and N-**acetyl glucosamine** units that are crosslinked through pentapeptide chains. The disruption of this structure leads to cell lysis; **peptidoglycan** biosynthesis is therefore an essential pathway and an important target for antibiotics research. The **enzyme** MurD catalyzes the addn. of D-glutamate to uridine diphosphate(**UDP**)-**MurNAc**-L-alanine in the biosynthesis of the **peptidoglycan** precursor **UDP-MurNAc** -pentapeptide. We have designed a mechanism-based phosphinate inhibitor of MurD (1). The synthesis and inhibitory activity of compd. 1 and its precursors will be discussed.

L17 ANSWER 19 OF 25 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 94227061 MEDLINE
DOCUMENT NUMBER: 94227061
TITLE: The glutamate racemase activity from Escherichia coli is regulated by **peptidoglycan** precursor **UDP** -N-acetylmuramoyl-L-alanine.
AUTHOR: Doublet P; van Heijenoort J; Mengin-Lecreulx D
CORPORATE SOURCE: Laboratoire des Enveloppes Bacteriennes et des Peptides, Unite de Recherche Associee 1131 du Centre National de la Recherche Scientifique, Universite Paris-Sud, Orsay, France.
SOURCE: BIOCHEMISTRY, (1994 May 3) 33 (17) 5285-90.
Journal code: A0G. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199408

AB The murI gene product of Escherichia coli was recently identified as the glutamate racemase activity which catalyzes the formation of D-glutamic acid, one of the essential components of **bacterial** cell-wall **peptidoglycan** [Doublet et al. (1993) J. **Bacteriol**. 175, 2970-2979]. We here describe the purification to homogeneity and the kinetic properties of this **enzyme**. In vitro, the glutamate racemase activity shows an absolute requirement for **UDP** -N-acetylmuramoyl-L-alanine (**UDP-MurNAc**-L-Ala), the substrate of the D-glutamic acid-adding **enzyme** which catalyzes the subsequent step in the pathway for **peptidoglycan** synthesis. The affinity of the **enzyme** for this activator is particularly high (KD = 4 microM) and specific, since no other **peptidoglycan** precursor from **UDP-GlcNAc** to **UDP-MurNAc**-pentapeptide is an effector. Minor chemical modifications of the **UDP-MurNAc**-L-Ala molecule, such as the reduction of the uracyl moiety, suppress its activating effect. This specific in vitro requirement most likely represents the physiological mechanism which regulates the activity of the glutamate racemase in vivo. It adjusts the formation of D-glutamic acid to the requirements of **peptidoglycan** synthesis and avoids an excessive racemization of the intracellular pool of L-glutamic acid.

TI The glutamate racemase activity from Escherichia coli is regulated by **peptidoglycan** precursor **UDP**-N-acetylmuramoyl-L-alanine.

AB . . . recently identified as the glutamate racemase activity which catalyzes the formation of D-glutamic acid, one of the essential components of **bacterial** cell-wall **peptidoglycan** [Doublet et al. (1993) J. **Bacteriol**. 175, 2970-2979]. We here describe the purification to homogeneity and the kinetic properties of this **enzyme**. In vitro, the glutamate racemase activity shows an absolute requirement for **UDP**-N-acetylmuramoyl-L-alanine (**UDP-MurNAc**-L-Ala), the substrate of the D-glutamic acid-adding **enzyme** which catalyzes the subsequent step in the pathway for **peptidoglycan** synthesis. The affinity of the **enzyme** for this activator is particularly high (KD = 4 microM) and specific, since no other **peptidoglycan** precursor from **UDP-GlcNAc** to **UDP-MurNAc** -pentapeptide is an effector. Minor chemical modifications of the **UDP-MurNAc**-L-Ala molecule, such as the reduction of the uracyl moiety, suppress its activating effect. This specific in vitro requirement most likely. . . regulates the activity of the glutamate racemase in vivo. It adjusts the formation of D-glutamic acid to the requirements of **peptidoglycan** synthesis and avoids an excessive racemization of the intracellular pool of L-glutamic acid.

RN **1941-66-8 (UDP-N-acetylmuramylalanine)**

L16 ANSWER 1 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Analogs of **UDP-MurNAc** peptides, assays and kits

L16 ANSWER 2 OF 64 USPATFULL
TI Auxiliary genes and proteins of methicillin resistant bacteria and antagonists thereof

L16 ANSWER 3 OF 64 USPATFULL
TI Methods of screening for compounds active on Staphylococcus aureus target genes

L16 ANSWER 4 OF 64 USPATFULL
TI Auxiliary genes and proteins of methicillin resistant bacteria and antagonists thereof

L16 ANSWER 5 OF 64 MEDLINE DUPLICATE 1
TI Assay for identification of inhibitors for bacterial MraY translocase or MurG transferase.

L16 ANSWER 6 OF 64 MEDLINE DUPLICATE 2
TI Chlorobiphenyl-desleucyl-vancomycin inhibits the transglycosylation process required for **peptidoglycan** synthesis in bacteria in the absence of dipeptide binding.

L16 ANSWER 7 OF 64 USPATFULL
TI Biosynthetic gene muri from Streptococcus pneumoniae

L16 ANSWER 8 OF 64 USPATFULL
TI Metabolic pathway assay

L16 ANSWER 9 OF 64 USPATFULL
TI Biosynthetic gene murD from streptococcus pneumoniae

L16 ANSWER 10 OF 64 USPATFULL
TI Biosynthetic gene murg from streptococcus pneumoniae

L16 ANSWER 11 OF 64 USPATFULL
TI **Peptidoglycan** biosynthetic gene murE from Streptococcus pneumoniae

L16 ANSWER 12 OF 64 USPATFULL
TI Biosynthetic gene DD1 from Streptococcus pneumoniae

L16 ANSWER 13 OF 64 USPATFULL
TI Biosynthetic gene ddl Streptococcus pneumoniae

L16 ANSWER 14 OF 64 USPATFULL
TI **Peptidoglycan** biosynthetic gene mur a from Strepococcus pneumoniae

L16 ANSWER 15 OF 64 USPATFULL
TI **Peptidoglycan** biosynthetic mure protein from streptocuccus pneumoniae

L16 ANSWER 16 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Substrate synthesis and activity assay for MurG

L16 ANSWER 17 OF 64 USPATFULL

TI **Peptidoglycan** biosynthetic mura protein from Streptococcus pneumoniae

L16 ANSWER 18 OF 64 USPATFULL
TI Murd protein method and kit for identification of inhibitors

L16 ANSWER 19 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Inhibitors of the bacterial cell wall biosynthesis enzyme Mur D.

L16 ANSWER 20 OF 64 MEDLINE DUPLICATE 3
TI The glutamate racemase activity from Escherichia coli is regulated by **peptidoglycan** precursor **UDP**-N-acetylmuramoyl-L-alanine.

L16 ANSWER 21 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Analysis of **peptidoglycan** precursors in vancomycin-resistant Enterococcus gallinarum BM4174

L16 ANSWER 22 OF 64 SCISEARCH COPYRIGHT 2000 ISI (R)
TI THE MURI GENE OF ESCHERICHIA-COLI IS AN ESSENTIAL GENE THAT ENCODES A GLUTAMATE RACEMASE ACTIVITY

L16 ANSWER 23 OF 64 SCISEARCH COPYRIGHT 2000 ISI (R)
TI **PEPTIDOGLYCAN** BIOSYNTHESIS IN ESCHERICHIA-COLI - VARIATIONS IN THE METABOLISM OF ALANINE AND D-ALANYL-D-ALANINE

L16 ANSWER 24 OF 64 MEDLINE DUPLICATE 4
TI The murG gene of Escherichia coli codes for the **UDP**-N-**acetylglucosamine**: N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-**acetylglucosamine** transferase involved in the membrane steps of **peptidoglycan** synthesis.

L16 ANSWER 25 OF 64 MEDLINE DUPLICATE 5
TI Analysis of murein and murein precursors during antibiotic-induced lysis of Escherichia coli.

L16 ANSWER 26 OF 64 MEDLINE DUPLICATE 6
TI Mureidomycin A, a new inhibitor of bacterial **peptidoglycan** synthesis.

L16 ANSWER 27 OF 64 MEDLINE DUPLICATE 7
TI Inhibition of **peptidoglycan** biosynthesis by ramoplanin.

L16 ANSWER 28 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Inhibition of **peptidoglycan** biosynthesis in Bacillus megaterium by daptomycin

L16 ANSWER 29 OF 64 MEDLINE
TI Correlation between the effects of fosfomycin and chloramphenicol on Escherichia coli.

L16 ANSWER 30 OF 64 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Correlation between the effects of fosfomycin and chloramphenicol on Escherichia coli.

L16 ANSWER 31 OF 64 MEDLINE DUPLICATE 8
TI Variations in **UDP**-N-**acetylglucosamine** and **UDP**-N-acetylmuramyl-pentapeptide pools in Escherichia coli after inhibition of protein synthesis.

L16 ANSWER 32 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Liposidomycin C inhibits phospho-N-acetylmuramyl-pentapeptide transferase in **peptidoglycan** synthesis of Escherichia coli Y-10

L16 ANSWER 33 OF 64 MEDLINE DUPLICATE 9
TI Determination of murein precursors during the cell cycle of Escherichia coli.

L16 ANSWER 34 OF 64 MEDLINE DUPLICATE 10
TI Intramembranal events in the biosynthesis of **peptidoglycan** in Gaffkya homari.

L16 ANSWER 35 OF 64 MEDLINE DUPLICATE 11
TI **Peptidoglycan** synthetic activities in membranes of Escherichia coli caused by overproduction of penicillin-binding protein 2 and rodA protein.

L16 ANSWER 36 OF 64 MEDLINE DUPLICATE 12
TI Isolation of differentiated membrane domains from Escherichia coli and Salmonella typhimurium, including a fraction containing attachment sites between the inner and outer membranes and the murein skeleton of the cell envelope.

L16 ANSWER 37 OF 64 MEDLINE DUPLICATE 13
TI Teicoplanin, a new antibiotic from Actinoplanes teichomyceticus nov. sp.

L16 ANSWER 38 OF 64 MEDLINE DUPLICATE 14
TI In vitro synthesis of **peptidoglycan** by spheroplasts of Proteus mirabilis grown in the presence of penicillin.

L16 ANSWER 39 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Intracellular accumulation of trehalose during streptomycin formation by Streptomyces griseus

L16 ANSWER 40 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Synthesis of **peptidoglycan** from externally supplied precursors by partly autolyzed cells of Bacillus subtilis W23

L16 ANSWER 41 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Cytoplasmic steps of **peptidoglycan** synthesis in E. coli K 12

L16 ANSWER 42 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI The complete sequence of murein synthesis in ether treated Escherichia coli

L16 ANSWER 43 OF 64 MEDLINE DUPLICATE 15
TI Biosynthesis of cadaverine-containing **peptidoglycan** in Selenomonas ruminantium.

L16 ANSWER 44 OF 64 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
TI Action of clofoctol on bacterial cell wall synthesis.

L16 ANSWER 45 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Inhibition of microbial cell wall synthesis by lipopeptin A

L16 ANSWER 46 OF 64 MEDLINE DUPLICATE 16
TI The site of inhibition of bacterial cell wall **peptidoglycan** synthesis by azureomycin B, a new antibiotic.

L16 ANSWER 47 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Dissociation and reconstitution of membranes synthesizing the **peptidoglycan** of Bacillus megaterium. A protein factor for the polymerization step

L16 ANSWER 48 OF 64 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 17
TI Amphomycin inhibits phospho-N-acetylmuramyl-pentapeptide translocase in **peptidoglycan** synthesis of Bacillus.

L16 ANSWER 49 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI The activities in vitro of DD-carboxypeptidase and LD-carboxypeptidase of Gaffkya homari during biosynthesis of **peptidoglycan**

L16 ANSWER 50 OF 64 MEDLINE DUPLICATE 18

TI Biosynthesis of spin-labeled **peptidoglycan**: spin-spin interactions.

L16 ANSWER 51 OF 64 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 19
TI Studies on bacterial cell wall inhibitors. II. Inhibition of **peptidoglycan** synthesis in vivo and in vitro by amphomycin.

L16 ANSWER 52 OF 64 MEDLINE
TI Steric effects on penicillin-sensitive **peptidoglycan** synthesis in a membrane-wall system Gaffkya homari.

L16 ANSWER 53 OF 64 MEDLINE
TI Biosynthesis of **peptidoglycan** in Staphylococcus aureus: incorporation of the Nepsilon-Ala-Lys moiety into the peptide subunit of nascent **peptidoglycan**.

L16 ANSWER 54 OF 64 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 20
TI A rapid and simple procedure for the preparation of the two bacterial cell
wall **peptidoglycan** nucleotide precursors labeled in their amino sugars.

L16 ANSWER 55 OF 64 MEDLINE DUPLICATE 21
TI [Study of the formation of N-glycolylmuramic acid from Nocardia asteroides
(author's transl)].
Etude de la formation de l'acide N-glycolylmuramique du peptidoglycane de Nocardia asteroides.

L16 ANSWER 56 OF 64 MEDLINE DUPLICATE 22
TI Chemical structure of the **peptidoglycan** of Vibrio parahaemolyticus A55 with special reference to the extent of interpeptide cross-linking.

L16 ANSWER 57 OF 64 MEDLINE DUPLICATE 23
TI Biosynthesis of **peptidoglycan** in Gaffkya homari. The mode of action of penicillin G and mecillinam.

L16 ANSWER 58 OF 64 MEDLINE DUPLICATE 24
TI Biosynthesis of **peptidoglycan** in Gaffkya homari. The incorporation of **peptidoglycan** into the cell wall and the direction of transpeptidation.

L16 ANSWER 59 OF 64 MEDLINE DUPLICATE 25
TI Pyruvate-uridine diphospho-N-**acetylglucosamine** transferase. Purification to homogeneity and feedback inhibition.

L16 ANSWER 60 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Control of synthesis of bacterial cell walls. Interaction in the synthesis of nucleotide precursors

L16 ANSWER 61 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Biosynthesis of the **peptidoglycan** of bacterial cell walls. XXII. Activation of D-aspartic acid for incorporation into **peptidoglycan**

L16 ANSWER 62 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Biosynthesis of **peptidoglycan** by a cell wall preparation of Staphylococcus aureus and its inhibition by penicillin

L16 ANSWER 63 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Biosynthesis of the **peptidoglycan** of bacterial cell walls. IV. Incorporation of glycine in Micrococcus lysodeikticus

L16 ANSWER 64 OF 64 CAPLUS COPYRIGHT 2000 ACS
TI Biosynthesis of the **peptidoglycan** of bacterial cell walls. II.

Phospholipid carriers in the reaction sequence

L16 ANSWER 6 OF 64 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 2000141264 MEDLINE
DOCUMENT NUMBER: 20141264
TITLE: Chlorobiphenyl-desleucyl-vancomycin inhibits the transglycosylation process required for **peptidoglycan** synthesis in bacteria in the absence of dipeptide binding.
AUTHOR: Goldman R C; Baizman E R; Longley C B; Branstrom A A
CORPORATE SOURCE: Incara Research Laboratories, 8 Cedar Brook Drive, Cranbury, NJ 08512, USA.. rgoldman@irl.incara.com
SOURCE: FEMS MICROBIOLOGY LETTERS, (2000 Feb 15) 183 (2) 209-14. Journal code: FML. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200006
ENTRY WEEK: 20000602

AB Novel glycopeptide analogs are known that have activity on vancomycin resistant enterococci despite the fact that the primary site for drug interaction, D-ala-D-ala, is replaced with D-ala-D-lactate. The mechanism of action of these compounds may involve dimerization and/or membrane binding, thus enhancing interaction with D-ala-D-lactate, or a direct interaction with the transglycosylase enzymes involved in **peptidoglycan** polymerization. We evaluated the ability of vancomycin (V), desleucyl-vancomycin (desleucyl-V), chlorobiphenyl-vancomycin (CBP-V), and chlorobiphenyl-desleucyl-vancomycin (CBP-desleucyl-V) to inhibit (a) **peptidoglycan** synthesis in vitro using **UDP**-muramyl-pentapeptide and **UDP**-muramyl-tetrapeptide substrates and (b) growth and **peptidoglycan** synthesis in vancomycin resistant enterococci. Compared to V or CBP-V, CBP-desleucyl-V retained equivalent potency in these assays, whereas desleucyl-V was inactive. In addition, CBP-desleucyl-V caused accumulation of N-**acetylglucosamine**-beta-1, 4-**MurNAc**-pentapeptide-pyrophosphoryl-undecaprenol (lipid II). These data show that CBP-desleucyl-V inhibits **peptidoglycan** synthesis at the transglycosylation stage in the absence of binding to dipeptide.

TI Chlorobiphenyl-desleucyl-vancomycin inhibits the transglycosylation process required for **peptidoglycan** synthesis in bacteria in the absence of dipeptide binding.

AB . . . involve dimerization and/or membrane binding, thus enhancing interaction with D-ala-D-lactate, or a direct interaction with the transglycosylase enzymes involved in **peptidoglycan** polymerization. We evaluated the ability of vancomycin (V), desleucyl-vancomycin (desleucyl-V), chlorobiphenyl-vancomycin (CBP-V), and chlorobiphenyl-desleucyl-vancomycin (CBP-desleucyl-V) to inhibit (a) **peptidoglycan** synthesis in vitro using **UDP**-muramyl-pentapeptide and **UDP**-muramyl-tetrapeptide substrates and (b) growth and **peptidoglycan** synthesis in vancomycin resistant enterococci. Compared to V or CBP-V, CBP-desleucyl-V retained equivalent potency in these assays, whereas desleucyl-V was inactive. In addition, CBP-desleucyl-V caused accumulation of N-**acetylglucosamine**-beta-1, 4-**MurNAc**-pentapeptide-pyrophosphoryl-undecaprenol (lipid II). These data show that CBP-desleucyl-V inhibits **peptidoglycan** synthesis at the transglycosylation stage in the absence of binding to dipeptide.

CT *Antibiotics, Glycopeptide: PD, pharmacology
*Bacteria: DE, drug effects
*Bacteria: ME, metabolism
Dipeptides: ME, metabolism
Glycosylation
***Peptidoglycan: BI, biosynthesis**
*Vancomycin: AA, analogs & derivatives
Vancomycin: PD, pharmacology
CN 0 (chlorobiphenyl-desleucyl-vancomycin); 0 (Antibiotics, Glycopeptide); 0 (Dipeptides); 0 (**Peptidoglycan**)

L16 ANSWER 31 OF 64 MEDLINE DUPLICATE 8
ACCESSION NUMBER: 89255094 MEDLINE
DOCUMENT NUMBER: 89255094
TITLE: Variations in **UDP-N-acetylglucosamine** and **UDP**-N-acetylmuramyl-pentapeptide pools in Escherichia coli after inhibition of protein synthesis.
AUTHOR: Mengin-Lecreulx D; Siegel E; van Heijenoort J
CORPORATE SOURCE: Unite Associee 1131 du Centre National de la Recherche Scientifique, Biochimie Moleculaire et Cellulaire, Universite Paris-Sud, Orsay, France..
SOURCE: JOURNAL OF BACTERIOLOGY, (1989 Jun) 171 (6) 3282-7. Journal code: HH3. ISSN: 0021-9193.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198909

AB The pool levels of the nucleotide precursors of **peptidoglycan** were analyzed after inhibition of protein synthesis in various Escherichia coli strains. In all cases **UDP-N-acetylglucosamine** (**UDP-GlcNAc**) and **UDP**-N-acetylmuramyl-pentapeptide (**UDP-MurNAc**-pentapeptide) cell pools increased upon treatment with chloramphenicol or tetracycline. Similar results were observed after the treatment of K-12 strains with valine. Since the intermediate nucleotide precursors did not accumulate after the arrest of protein synthesis and since a feedback mechanism was unlikely, the increases of the **UDP-MurNAc**-pentapeptide pool appeared as a consequence of that of the **UDP-GlcNAc** pool by the unrestricted functioning of the intermediate steps of the pathway. The highest increase (sixfold) of **UDP-GlcNAc** was observed with strain K-12 HfrH growing in minimal medium and treated with chloramphenicol. When a pair of isogenic Rel+ and Rel- strains were considered, both the **UDP-GlcNAc** and **UDP-MurNAc**-pentapeptide pools increased upon treatment with chloramphenicol or valine. However, the **UDP-GlcNAc** pool of the Rel+ strain was at a high natural level, which increased only moderately (20%) after the addition of valine. The increase of the **UDP-GlcNAc** pool after the various treatments could be due to an effect on some upstream step by an unknown mechanism. The possible correlations of the variations of the precursor pools with the rate of synthesis and extent of cross-linking of **peptidoglycan** were also considered.

TI Variations in **UDP-N-acetylglucosamine** and **UDP**-N-acetylmuramyl-pentapeptide pools in Escherichia coli after inhibition of protein synthesis.

AB The pool levels of the nucleotide precursors of **peptidoglycan** were analyzed after inhibition of protein synthesis in various Escherichia coli strains. In all cases **UDP-N-acetylglucosamine** (**UDP-GlcNAc**) and **UDP-N**-acetylmuramyl-pentapeptide (**UDP-MurNAc**-pentapeptide) cell pools increased upon treatment with chloramphenicol or tetracycline. Similar results were observed after the treatment of K-12 strains with. . . did

not accumulate after the arrest of protein synthesis and since a feedback mechanism was unlikely, the increases of the **UDP-MurNAc**-pentapeptide pool appeared as a consequence of that of the **UDP-GlcNAc** pool by the unrestricted functioning of the intermediate steps of the pathway. The highest increase (sixfold) of **UDP-GlcNAc** was observed with strain K-12 HfrH growing in minimal medium and treated with chloramphenicol. When a pair of isogenic Rel+ and Rel- strains were considered, both the **UDP-GlcNAc** and **UDP-MurNAc**-pentapeptide pools increased upon treatment with chloramphenicol or valine. However, the **UDP-GlcNAc** pool of the Rel+ strain was at a high natural level, which increased only moderately (20%) after the addition of valine. The increase of the **UDP-GlcNAc** pool after the various treatments could be due to an effect on some upstream step by an unknown mechanism. The possible correlations of the variations of the precursor pools with the rate of synthesis and extent of cross-linking of **peptidoglycan** were also considered.

CT Check Tags: Support, Non-U.S. Gov't
*Bacterial Proteins: BI, biosynthesis
Chloramphenicol: PD, pharmacology
*Escherichia coli: ME, metabolism
***Peptidoglycan: BI, biosynthesis**
Tetracycline: PD, pharmacology
***Uridine Diphosphate N-Acetylglucosamine: ME, metabolism**
Uridine Diphosphate N-Acetylmuramic Acid: AA, analogs & derivatives
*Uridine Diphosphate N-Acetylmuramic Acid: ME, metabolism
*Uridine Diphosphate. . .

RN **16124-22-4 (UDP-N-acetylmuramic acid pentapeptide)**; **528-04-1 (Uridine Diphosphate N-Acetylglucosamine)**; 56-75-7 (Chloramphenicol); 60-54-8 (Tetracycline); 7004-03-7 (Valine)

L16 ANSWER 38 OF 64 MEDLINE DUPLICATE 14
ACCESSION NUMBER: 85096096 MEDLINE
DOCUMENT NUMBER: 85096096
TITLE: In vitro synthesis of **peptidoglycan** by spheroplasts of Proteus mirabilis grown in the presence of penicillin.
AUTHOR: Martin H H
SOURCE: ARCHIVES OF MICROBIOLOGY, (1984 Nov) 139 (4) 371-5. Journal code: 7YN. ISSN: 0302-8933.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198504

AB Spheroplasts of the unstable L-form of Proteus mirabilis with fragile, shape defective cell walls grown in medium containing 120 mg/l penicillin G and then killed and permeabilized by ether treatment, were capable of in vitro synthesis of **peptidoglycan** from the precursors **UDP-GlcNAc** and **UDP-MurNAc**-L-Ala-D-Glu(ms-A2pm-D-Ala-D-Ala). The in vitro **peptidoglycan** was extensively peptide-crosslinked, indicating a continuing function of **peptidoglycan** transpeptidase in the spheroplasts. The seven penicillin-binding proteins (PBPs) of P. mirabilis with their functions as multiple **peptidoglycan** transpeptidases were shown to be saturated in the spheroplasts and thereby functionally inactivated by the penicillin of the growth medium to a very different degree. Complete or almost complete saturation occurred with the PBPs 1A, 1B, and 3, for which functions as indispensable transpeptidases in Escherichia coli have been postulated. In contrast, PBPs 5 and 6 were not saturated in the L-form spheroplasts. Transpeptidase function has been described previously in PBP 5 of P. mirabilis. The working hypothesis is proposed that synthesis of

the functionally defective **peptidoglycan** of L-form spheroplasts in the presence of penicillin takes place with transpeptidase function of PBP 5.

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CT Check Tags: In Vitro; Support, Non-U.S. Gov't
Acetylation
Carrier Proteins: AN, analysis
Muramoylpentapeptide Carboxypeptidase: AN, analysis
*Penicillins: PD, pharmacology
***Peptidoglycan: BI, biosynthesis**
Peptidyl Transferases: ME, metabolism
*Proteus mirabilis: ME, metabolism
Spheroplasts: ME, metabolism

L16 ANSWER 51 OF 64 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.DUPLICATE 19
ACCESSION NUMBER: 78105005 EMBASE
DOCUMENT NUMBER: 1978105005
TITLE: Studies on bacterial cell wall inhibitors. II. Inhibition of **peptidoglycan** synthesis in vivo and in vitro by amphomycin.
AUTHOR: Tanaka H.; Iwai Y.; Oiwa R.; et al.
CORPORATE SOURCE: Kitasato Univ., Tokyo, Japan
SOURCE: Biochimica et Biophysica Acta, (1977) 497/3 (633-640). CODEN: BBACAQ
COUNTRY: Netherlands
DOCUMENT TYPE: Journal
FILE SEGMENT: 037 Drug Literature Index
004 Microbiology
030 Pharmacology
029 Clinical Biochemistry
LANGUAGE: English

AB Amphomycin has been reported to be a selective inhibitor of cell wall **peptidoglycan** synthesis in Bacillus cereus T. Investigations were carried out to clarify the target of amphomycin. Amphomycin (10 .mu.g/ml) lysed growing cells of B. cereus T, and inhibited **peptidoglycan** synthesis, accompanied by accumulation of uridine diphosphate-N-acetylmuramyl (**UDP-MurNAc**) peptides. The nucleotide precursors that accumulated in cells of Staphylococcus aureus FDA 209P in the presence of amphomycin were identified as **UDP-MurNAc**-L-Ala-D-Glu-L-Lys-D-Ala-D-Ala, **UDP-MurNAc**-L-Ala and **UDP-MurNAc**. In the experiments using a particulate enzyme system of Bacillus megaterium KM, amphomycin inhibited the polymerization of **UDP-MurNAc**-L-Ala-D-Glu-meso-diaminopimelic acid-D-Ala-D-Ala (**UDP-MurNAc**-pentapeptide) and **UDP-N-acetylglucosamine**, and also inhibited the formation of lipid intermediates, but did not inhibit the cross-linking, the last step of **peptidoglycan** synthesis. Unlike bacitracin, amphomycin did not lyse protoplasts of B. megaterium KM. It is

concluded that the site of action of amphomycin is the formation of **MurNAc**-(pentapeptide)-P-P-lipid from **MurNAc**-pentapeptide and undecaprenol (lipid) phosphate.

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CT Medical Descriptors:
*bacillus cereus
*bacillus megaterium
*bacterial cell wall
*diaminopimelic acid h 3
*staphylococcus aureus
theoretical study
in vitro study
microorganism
*amfomycin
***peptidoglycan**
radioisotope

RN (amfomycin) 1402-82-0, 8057-36-1; (**peptidoglycan**) 9047-10-3

L16 ANSWER 60 OF 64 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1974:79945 CAPLUS
DOCUMENT NUMBER: 80:79945
TITLE: Control of synthesis of bacterial cell walls. Interaction in the synthesis of nucleotide precursors
AUTHOR(S): Anderson, Raymond G.; Douglas, L. Julia; Hussey, Helen; Baddiley, James
CORPORATE SOURCE: Microbiol. Chem. Res. Lab., Univ. Newcastle-upon-Tyne, Newcastle-upon-Tyne, Engl.
SOURCE: Biochem. J. (1973), 136(4), 871-6
CODEN: BIJOAK
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Enzymes involved in forming **peptidoglycan** and teichoic acid are regulated by precursors of the alternative product. In sol. exts of Bacillus licheniformis ATCC 9945 phosphoenolpyruvate **UDP**-N-**acetylglucosamine** enolpyruvyltransferase was inhibited by **UDP-acetylmuramylpentapeptide**, **UDP**-N-**acetylglucosamine** pyrophosphorylase was inhibited by the pentapeptide and, in a concn.-dependent manner, by CDP-glycerol, and CDP-glycerol pyrophosphorylase was inhibited by the pentapeptide and CDP-glycerol and stimulated by **UDP**-N-**acetylglucosamine**.

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ST Bacillus wall formation regulation; enzyme regulation **peptidoglycan** formation; teichoic acid formation regulation; cell wall formation regulation

IT Bacillus licheniformis
(**peptidoglycan** and teichoic acid formation by, control of)

IT 9041-38-7
RL: FORM (Formation, nonpreparative)
(formation of, **peptidoglycan** precursors control of, in Bacillus licheniformis)